

## Disarmament's month in New York

A RARE opportunity for disarmament to occupy the centre of the world stage comes up shortly with the convening of a special session of the United Nations General Assembly devoted to disarmament. From 23 May to 28 June New York will be host to a conference (originally proposed by Yugoslavia many years ago) on a subject which has almost universal popular support in theory but which has only very modest successes to its name in practice. And not only is it difficult to stimulate disarmament, it is almost equally difficult to agree even on how to talk about it. Yet there is optimism that this conference will have some beneficial and long-lasting effects.

The problem with disarmament talks is the enormous range of national viewpoints. There are nations which believe that the only real subject to talk about is general and complete disarmament. There are nations that want no obstacles to modernising their own arsenals. There are nuclear nations worried about some anti-nuclear resolution catching the conference's imagination. There are non-nuclear nations concerned at the possibility of nuclear attack. There are nations worried about the behaviour of their immediate neighbours. There are nations that would like to link disarmament with development. There are non-governmental organisations with their own particular axes to grind. And over all this hovers the general understanding that the conference must come to a consensus on major issues—disarmament matters that have to be voted on are most unlikely to carry much conviction. One could be forgiven for seeing all this as a recipe for a meaningless gathering leading to nothing of substance.

Certainly there is unlikely to be tangible progress towards specific treaties in New York. Negotiations in the pipeline on a comprehensive test ban, a strategic arms limitation agreement or a mutual force reduction are too delicate and complex to profit by exposure to a vast assembly of nations—or so it is argued. But arms control and disarmament is a slow-moving scene most of the time, so the more perceptive amongst those going to New York are more likely to be looking as far ahead as the year 1990 and asking whether it might be reasonable to place a few milestones on the road between now and then. For up to the present the emergence of such treaties as there have been has been a piecemeal affair, depending little on what the community of nations really needed at an particular time or on what should be a logical next step, but almost entirely on what a few nations were prepared to give. In short, disarmament has really had no long-term agenda. The hope then is that out of the New York meeting may spring

a common resolve to rectify this anomaly and devise a widely acceptable programme of action.

To get such a disarmament strategy on the road, however, will require more than just goodwill. There are three areas in particular where much hard work will be needed. The first is in research: investigations into the real extent of the trade in conventional arms; experimental studies of how disarmament might be linked to development; design and analysis of verification schemes for disarmament measures, and so on. For this the United Nations might be urged to set up its own disarmament agency in the same way that it has agencies for health, food and meteorology.

The second area needing attention is in the field of information. There are two facets to this—international information exchange on armaments matters, and the stimulation of public interest in disarmament. One of the most effective means of paving the way towards disarmament is for nations to provide more detailed information to each other on the extent of their military operations, research and development programmes and so on. Certainly the bilateral exchanges between the two superpowers of the past few years, exchanges which some years previously would have been regarded as high treason, have slowly helped to build confidence that neither side will misread the other's intentions—there is clearly scope for more of this. On the other hand greater public involvement in disarmament matters is also much to be desired. Many, but not all Western countries have done very little to invite those outside a small circle to think constructively on this subject; the United Kingdom has a poor record on this, but improvement may be on the way.

Thirdly, the United Nations machinery for disarmament needs careful re-appraisal. The Geneva-based Conference of the Committee on Disarmament, loosely coupled to the United Nations, has developed a reasonably good line in consensus politics, and is not as large (31 members) as to be subject to many of the problems of giant UN committees. On the other hand it is largely dominated by the superpowers who act as co-chairmen, and usually has to wait on superpower agreement before it can do anything profitable. In New York there are certain to be moves to cut down on this dominance, and to make the committee more closely responsive to UN wishes. As long as this does not mean a major expansion in numbers, the move is to be welcomed.

The next month in New York will get few headlines, and such agreements as may emerge may seem unspectacular in the extreme. But there is reason to believe that a step forward could well be taken. □



# Separate development for science

Ziauddin Sardar, an information consultant at the King Abdul Aziz University, Jeddah, Saudi Arabia, argues for scientific independence for developing countries.

NARENDRA SINGH's comments (9 March, page 112) on Michael J. Moravcsik's plea for "the voice of science of the Third World" (24 November, page 288) are a good illustration of the gulf between the concerned and aware scientists of the developed and developing countries. Moravcsik is concerned with "scientific development" (which sounds a little omniscient), "foreign students", "scientific communication channels" and "self-image"; Singh is concerned with "politico-economic content" of science and technology, "self-reliance" and "self-sufficiency". Moravcsik is optimistic about the forthcoming United Nations Conference on Science and Technology for Development (UNCSTD); Singh is lukewarm.

I agree with much of what Singh has to say, although I would argue strongly for 'isolation'. By isolation I mean the need for the developing countries to drop out of the "science development" race propagated by Moravcsik and others.

Singh's entire approach to UNCSTD is conditioned by a realisation that is slowly taking root in the scientific and technological institutions of many developing countries: there is something inherent in science and technology as it is practised in the West that will never allow the developing countries to stand on their own feet. Furthermore, this "something" has an ideological character; and it is certainly too deeply entrenched to be weeded out by a single UN conference.

After all, one may ask, what is so special about UNCSTD? The developing countries have the experience of four UNCTAD meetings to learn from. On all four occasions the developed world categorically refused to see the point of view of the developing countries. The UNCTAD meetings were concerned about economic injustice; the UNCSTD is concerned with the instruments that helped the creation of the injustice in the first place, and maintains the *status quo*.

There are strong signs that UNCSTD will meet the same fate as UNCTAD. The pre-organisation fiasco, the multi-national industrial lobby (which holds the monopoly of modern science and technology) persuading and forcing the US policy towards preserving their proprietary rights, the exclusion of any discussion of appropriate technologies *per se*, the emergence of 'north-south dialogue' - all indicate this direction. The stage is set not for a dialogue based on mutual self-respect, geared towards achieving positive results, but for a confrontation.

While the developing countries are forced into it, they do not desire a confrontation. On the ideological plane, they want a basic minimum standard of living and a modicum of economic activity for all their citizens, a sense of participation from all groups in society, and restraint on the concentration of economic power so that it does not become an instrument of cultural and political exploitation. On the psychological plane the developing countries desire to shake off the hurt and humiliation of their colonial past. Both these desires are interlinked; and both must be realised.

In this context, Singh's emphasis on self-reliance and self-sufficiency takes on a special meaning. The developing countries need to isolate themselves from western science and technology, perhaps even more so from the western 'development consultants'.

The principle of self-reliance and self-sufficiency requires making do with what one has and developing one's indigenous resources with one's own skills and effort. The developing countries must develop their own, *albeit* humble, less pretentious, style of science and technology. The emphasis should be on indigenous development stemming from rural cultures and the need for encouraging cultural authenticity. The traditional cultures must be protected from the onslaught of western patterns of consumption and those consumer goods that represent the omnipotence of technology (including 'Space 1999' and Bionic Woman!).

The 'republic of science' with its insistence on the international and universal character of scientific activity, the all-encompassing emphasis on the objectivity of science and its quest for truth, and the rejection of any type of imposition on the selection of research topic, has no role to play here. It is this very republic that in the name of objectivity and truth perpetuates the dependency trap.

Developing countries should reject all offers of western technological assistance. The acceptance of this aid acts against their two basic desires mentioned above. It encourages elitism and concentrates economic power in fewer and fewer hands. It generates a sense of indignity. It enforces western patterns of development, increases dependency, and enhances the prestige of western science and technology. Because foreign technology has been readily accessible in the past, there has been little pressure on the domestic scientific community to provide viable alternatives. The resulting inactivity has reduced the domestic scientific communities to mediocrity, and alienated the creative.

The practice of sending students for higher education to the West has perpetuated the technological dominance of the West. The 'been to's are misfits; they are unable to meet the indigenous requirements on the one hand, and generate a sense of inferiority in the local scientists on the other. Their hopes, aspirations and desires go against the dictates of self-reliance and self-sufficiency. They can only retain their position by identifying themselves with the western scientific community. One finds them zealously defending their (western) research interests and universalism of science. The national papers for UNCSTD will largely be prepared by such individuals; and, as such, they are hardly likely to be in the national self-interest.

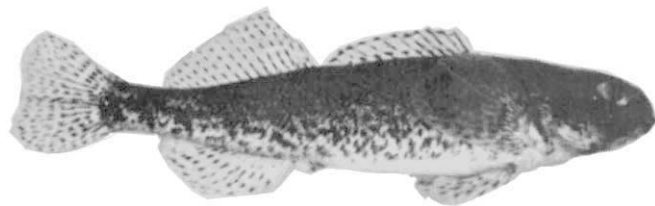
The Science Policy Research Unit at the University of Sussex has shown that less than 1% of the total research effort of the developed countries has any significance for the developing world. Under such circumstances, involvement in western scientific communication systems only distracts the scientists in the developing countries from the more pressing local issues. Hence there is a need for the developing countries to evolve their own network of scientific communications. Visiting scientists should go to centres of scientific activity in another developing country, rather than to the centres of the West.

Where some developed countries (such as Italy) are experiencing an enforced de-development, and others are hovering from crisis to crisis, a little isolation from western science and technology may not be a bad thing for the developing countries. Even the smaller and weaker amongst them which see technological and scientific aid as a stark necessity must feel a sense of discomfort at making demands from a position of weakness.

The equation, however, must be balanced. Both self-reliance and dignity begin from the self. The developing countries must take a searching look at themselves, develop their own capabilities and meet their own needs.

Meanwhile, if UNCSTD brings positive results, let us all share the glad tidings! □





## The fish that roared

David Dickson describes how attempts to save a fish—the snail darter—are threatening the Endangered Species Act

THE Tennessee Valley Authority agreed last week to revise its previous intransigence on plans to complete a \$120 million dam that could involve the extinction of a rare species of fish, the 3-inch snail darter.

Two members of the TVA board, chairman Aubrey Wagner and David S. Freeman, agreed to consult with the Department of the Interior over ways of preserving the fish.

The snail darter has become a national celebrity since its discovery in 1973, and the realisation that its only known habitat in the Little Tennessee River was threatened by the Tellico Dam, then 90% complete. Under the Endangered Species Act of 1973, federal agencies cannot carry out any project that would destroy the natural habitat of a species listed as endangered by the Interior Department.

Since the act was signed, the department has been consulted over 5,000 times by various agencies on proposed projects. Only in three cases has there been major disagreement: the Mississippi sand-hill crane (threatened by a state highway interchange) the Furbish lousewort (a small plant) and most notably the snail darter.

Last year an appeal court rejected a request from the federally-owned TVA that it be allowed to proceed with completing the dam. The case was heard in the Supreme Court last month, and judgement is expected in mid June.

The case has become a classic confrontation between environmentalists and corporate interests (a struggle further highlighted two weeks ago when Robert Strauss, President Carter's economic trouble-shooter, raised a furore by claiming that environmental measures were inflationary).

Supporters of the TVA—and indeed of other large-scale construction projects throughout the country which are similarly threatened—argue that they are facing an “environmental extremism” which ignores the important economic benefits of such projects.

Opponents of TVA put forward two main arguments. The first is that, faced with the possibility that up to 20% of known species could disappear by the end of the century, giving way

on the snail darter would provide a dangerous precedent for other, more significant cases.

The second argument is that each species represents a unique genetic resource that could eventually prove of medical or scientific benefit to man. Michael Beans, for example, chairman of the Environmental Defense Fund's Wildlife Programme, points to the example of the horse-shoe crab, whose blood was recently shown to be a highly efficient indicator of the presence of bacterial endotoxins in intravenous fluids.

Differences of opinion also emerged—somewhat to the Supreme Court's embarrassment—within the administration. Interior Secretary Cecil D. Andrus has stuck closely to the brief given to his Department by the Endangered Species Act, which allows no exemption from attempts to preserve threatened species.

However, Attorney General Griffin Bell, making his one (and so far only) appearance in the Supreme Court during the present administration with a bottled snail darter in his hand, argued strongly that the TVA should be allowed to complete the Tellico dam.

The new willingness of the TVA to discuss the preservation of the snail darter is therefore a significant shift from the position argued by last month's court hearing (and has largely been made possible by the resignation last week of the board's third member, William L. Jenkins, quoting frustration over the dam's completion as one of his reasons).

It also makes it easier for the Supreme Court to suggest, as a compromise formula, that no firm decision be made until the outcome is known of current attempts to establish the snail darter in an alternative nearby habitat in the Hiwassee River.

However, if this does turn out to be the Court's judgement, environmentalists claim that it will be “ducking the main issue”, namely whether the administration is prepared to stand firm on the Endangered Species Act and interpret it rigidly, or whether exemptions are to be permitted.

Already Senator John C. Culver of

Iowa and Senator Howard H. Baker of Tennessee have introduced a bill into Congress which would amend the act by setting up a seven-person committee which could, in cases of “irreconcilable conflict” between a federal agency and the Interior Department, judge on a majority basis whether an exemption from the act should be permitted.

The bill was reported out of the Senate committee on environment and public works at the end of last week, and stands a good chance of being accepted on the floor of the Senate (although it may face stiffer opposition in the House of Representatives, members of which are coming up for re-election in the autumn).

Supporters of the bill, admitting that it is largely a response to concern generated by the snail darter case, claim that it is nevertheless an attempt to protect, rather than dismantle, the main provision of the Endangered Species Act.

Opponents, however, argue that it would effectively create a committee which, for the first time in history, would have the power consciously to condemn a species to extinction.

Nor does the impact of the snail darter debate stop there. In addition to Mr Jenkin's resignation, Mr Wagner is also due to retire as chairman of the TVA board today (18 May); and President Carter has to select two new commissioners in addition to Mr Freeman, whom he appointed last year and who is expected to take over as chairman.

The President has, in the past, promised to make TVA into a showcase for his ideas about community development, and in particular a proving ground for energy innovation (such as solar power); Mr Freeman has been an outspoken advocate of such an approach, but has previously been speaking from a minority position.

However, nominations to the board must be approved by Congress. And local Tennessee Senators and Representatives are also concerned to see the continuance of one of the intended victims of Carter's energy policies, the Clinch River fast breeder reactor project.

Many eyes are therefore on the President to see which way he will turn in making his nominations. Except, perhaps, those of the snail darter itself, now in the middle of the mating season and with other things, no doubt, on its mind. □



## Yurii Orlov suffers two trials

"DISSIDENCE is an occupational hazard of the scientist!" This was the message delivered last autumn to the Venice Biennale on cultural dissent by Valentin Turchin, a cyberneticist, founder-member of the (illicit) Moscow group of Amnesty International and currently a political expellee.

The same theme was re-iterated yet again this week by the 'Orlov Hearing' at the Institute of Physics in London. This was a mock-trial in which John Macdonald, Q.C. presented the case for the defence simultaneously with the official trial in Moscow of Yurii Orlov, formally on a charge of slandering the Soviet state, *de facto* for his membership of the Helsinki monitoring group.

The case of Orlov—a corresponding member of the Academy of Sciences of the Armenian SSR—has attracted considerable attention throughout the world, not least because he is the highest-ranking Soviet scientist to be brought to trial on a political charge since the death of Stalin.

According to Mr Macdonald, a sufficient defence in Soviet law would be constituted by a proof that the data circulated in the information bulletins



of Orlov's 'Helsinki group' was true. Hence much of the evidence dealt with such abstruse points as the rights of the Crimean Tartars to return to their historic homeland, the rights of poor Ukrainian workers to emigrate to Canada, and the rights of a Baptist or Pentecostal to educate his children in his own faith—all of which are technically guaranteed either by the Soviet constitution, or by the various international human rights conventions to which the Soviet Union is a signatory.

However, as Turchin pointed out in Venice, it is standard scientific practice to apply the criteria of truth and consistency to all that one observes. It is not surprising, therefore that Orlov and his associates in Moscow (who included dissident Anatolii Shcharanskii, himself now awaiting trial, and Madame Elena Bonner, the wife of Academician

Andrei Sakharov) were concerned not with this or that particular abuse, but the whole concept of human rights.

The particular abuses reported at the hearing are, alas, now commonplace; political dissenters in psychiatric hospitals tied to their beds and denied the "privilege" of using the W.C., subjected to "reforming" doses of psychotropic drugs, or, as in the recent case of Nikolaev, refused a visit from next of kin due to foreign protests.

"Orlov's most characteristic trait," said Andrei Amalrik, co-founder of the Helsinki Monitoring Group, "is that he is not an emotional person. Before he makes a judgment, he verifies the facts thoroughly."

According to reliable reports, Orlov, while in pre-trial custody, has completed two scientific papers, one on symbolic logic, and one on elementary particle physics.

**Vera Rich**

● Message from Dr Robert Kates, Chairman of the Human Rights Committee of the US National Academy of Science:

"Many scientists around the world will be watching carefully the outcome of the trial commencing today for signs that the Soviet legal process is responsive to its own constitutional guarantees of freedom of thought and opinion. Yurii Orlov is a distinguished Soviet scientist who has contributed significantly to his country's inestimable progress in science. One only hopes that he will be dealt with fairly and justly in the proceedings taking place today. We will be watching . . ." □

## Re-usable satellite the future for UK space research?

If Britain's space science lobby has its way, some of Britain's space-borne experiments in the 1980s will be carried by an American re-usable satellite. The satellite, the 'multimission modular space craft (MMS)', is now under development at the Goddard Space Center in the US.

The space shuttle will spawn MMS satellites, leaving them aloft when the shuttle returns to Earth. Its attractions to Britain's space scientists are that it is cheap, re-usable, and powerful. It can be bought in kit form for \$9m and assembled at home; and the three large modules of the MMS—providing power, attitude control, and data handling—can cope with 3,000 kg of astronomical instrumentation.

According to Professor Ken Pounds, X-ray astronomer of Leicester University, the MMS should have arcsec pointing accuracy and could serve Britain for some 15 or 20 years. In that time, three or four experiments could be launched around the same MMS package. The first experiment, including the whole MMS and instrument costs might amount to £10m. Discounting over five years—the time

the experiment might last—the costs amount to £2m a year. This is "no more than the old Skylab programme" said Pounds, and considerably less than the present British spending in ESA, the European Space Agency.

The cost of Britain's participation in ESA amounts to an £8m subscription, paid through the Science Research Council, and a current £5 to £6m spent on research projects. In addition another £30m is paid by the Department of Industry which contributes to ESA applications, and to work on Spacelab, communications, the MAROTS satellite, Ariane launch technology, and the Uhuru launch base.

A number of European space scientists are becoming dissatisfied with ESA. And in the UK, where space science budgets are steadily shrinking against a fixed ESA subscription, the dissatisfaction is growing. Next year's UK6 will be the last British research satellite. "If in two or three years we had only £4m to spend on research projects, you would get a 100% UK vote to come out of ESA", Pounds said last week.

"ESA runs perhaps one mission for us every two years. There are five scientific disciplines and that leaves each one with a flight every 10 years. That's not value for money". Taking small amounts of space on US satellites helps out, says Pounds, but competition is intense and there is a natural bias towards US experiments. The MMS is the only way that Britain can keep up with world space science in the 1980s, thinks Pounds. "Otherwise we will be left with the tag ends of other people's missions."

● The Appleton Laboratory at Slough, which manages Britain's satellites (including UK5 at present and UK6 to follow), is to be moved to the Rutherford Laboratory some 40 miles away, if the recommendations of a Science Research Council rationalisation committee are accepted. Appleton has done little satellite engineering; and the Rutherford needs to find work for its accelerator engineers when Nimrod closes on 6 June. The result may be a team "which can get us more of the ESA action" said Professor Pounds last week.

**Robert Walgate**

## Windscale go-ahead; waste committee set up

BRITISH Nuclear Fuels Limited have won the final stage in their battle to build a £600 m expanded nuclear fuel reprocessing plant in North-West England. On Monday the House of Commons gave its approval, by a vote of 224 to 80, to the instrument which gives BNFL the right to begin building a thermal oxide reprocessing plant (THORP) capable of handling 1,200 tonnes per year of oxide fuel. BNFL's first step will be to construct cooling ponds for incoming fuel rods, and to make a detailed design of the plant. The first bricks of THORP will not be laid for perhaps as long as 5 years, and the plant will not be operational until the 1990s.

This gap was noted by a number of MPs. Mr Michael Heseltine, opposition spokesman on the issue, said "This is not an irreversible decision . . . this house should have a final opportunity to look at the facts after 3 or 4 years have passed". The opposition supported the present order but the house "should be kept informed".

Peter Shore, Secretary of State for the Environment, appeared to agree. He announced the setting up of a "radioactive waste management committee" (as recommended by the Flowers Commission on Environmental Pollution some years previously). The committee will report annually to the House. It will be chaired by Sir Denys Wilkinson, Vice-Chancellor of the University of Sussex and ex-head of the Department of Nuclear Physics at Oxford. Members of the committee have not yet been named but they will contain "a majority of nine or ten independent people with expertise in the relevant areas," said Mr Shore. There will also be representatives of BNFL, the United Kingdom Atomic Energy Authority, the electricity generating boards, and trade unions.

Such are the vagaries of parliamentary procedure that Nigel Forman, one of the best-informed Tory MPs on energy matters, was given little chance to speak in the debate. The house has thus left unheard a strong criticism of the economics of THORP. As the principal argument for THORP to be heard in the House was that it had enormous economic value to Britain, the loss of Forman's speech was significant. Robin Cook based his criticism on the inadequacies of the Parker report, which he doubted would stand the test of the next 3 or 4 years, and on proliferation, which he insisted THORP would increase by its example. "If we have a reprocessing plant, how can we object to Brazil or Pakistan having one too?" **Robert Walgate**

## NIH may loosen recombinant DNA research guidelines

MAJOR changes are expected to be announced soon in both the administration and the content of guidelines established by the National Institutes of Health for the conduct of research using recombinant DNA techniques.

The existing guidelines were laid down in 1976, and a draft of proposed revisions was published last summer by the director of NIH, Dr Donald Fredrickson, following evidence which, according to many observers, showed the guidelines to be over-restrictive.

Dr Fredrickson is now about to publish a further version of the proposed revision, with in many cases even lower safety requirements than suggested last year. This follows the acceptance of a number of recommendations put by Dr Fredrickson to the NIH's Recombinant DNA Molecule Programme Advisory Committee, which was responsible for drawing up the original version of the guidelines.

One important recommendation is that responsibility for the safe conduct of research using recombinant DNA techniques should be devolved from the NIH to the research institutions themselves, where it would be the responsibility of an institutional biohazard committee.

Such a committee would be empowered to approve the safety precautions proposed for a particular experiment. Rather than the research worker requiring to have a Memorandum of Understanding and Agreement accepted by the NIH, it will merely be necessary for the biohazards committee to provide notification that an experiment has been approved, and to file the MUA with the NIH.

In view of their greatly increased responsibility, the biohazards committees would include members of the public. (In a close vote, the advisory committee also suggested that employees' representatives should be included, but Dr Fredrickson has not yet indicated whether he is prepared to accept this.)

With regard to the content of the guidelines, the advisory committee accepted a recommendation from Dr Fredrickson to allow a substantial reduction in the safety requirements necessary to conduct work involving viruses.

This recommendation was based on the conclusions of a working party which met at NIH last month, as well as an EMBO workshop held in England last year. Containment levels for cloning viruses from warm-blooded vertebrates in *Escherichia coli*, for ex-

ample, would be reduced to the P2/EK1 combination of physical and biological containment; the existing proposal is to revise the level down to P4/EK1 or P3/EK2.

The advisory committee also approved the exemption from the guidelines of research involving certain recombinant DNA molecules which consist entirely of plasmid sequences from species that exchange DNA by known physiological processes, even though one or more of the segments may be a synthetic equivalent.

The committee approved for exemption a number of such experiments, making the exception of research involving *Bacteroides sp.* and *Haemophilus influenzae*.

In making his recommendations to the advisory committee, Dr Fredrickson emphasised the desirability of agreeing on alterations before the passage of legislation extending the guidelines to all research carried out using recombinant DNA techniques, including that in private industry.

However, on Capitol Hill the chances of legislation being passed within the near future are becoming increasingly remote. The House of Representatives has not set a date to debate HR11192, the bill reported out of Mr Harley Staggers' commerce committee over two months ago; and in the Senate there is still confusion about what the next step will be.

Senator Edward Kennedy, chairman of the health and scientific research subcommittee of the human resources committee, who had previously introduced his own version of the House bill, is now reported to feel that no new federal legislation may be necessary after all. Two weeks ago Kennedy wrote to Dr Joseph Califano, Secretary for Health, Education and Welfare, asking whether existing legislation—such as Section 361 of the Public Health Service Act which gives Califano control over protection against "communicable diseases"—may provide adequate legislative machinery.

The HEW has replied to Kennedy, however, that the administration is still keen to see the implementation of HR 11192, and that it does not consider Section 361, which contains no provision for federal preemption of state controls, to represent "appropriate authority" for achieving uniform regulation of recombinant DNA research. There is also uncertainty within the administration over whether the potential dangers can be classified as a communicable disease.

**David Dickson**





Swarm conditions, and the culprit in close-up (right)

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## Locusts: vigilance still needed

Camera Press

SUCH is the decline in surveying and reporting the activities of the desert locust (*Schistocerca gregaria*) that the precise significance of recent increased breeding of this dangerous insect cannot yet be estimated. It does seem that despite the somewhat alarming press releases put out earlier this year by FAO—which plays an overall, co-ordinating role in desert locust control—locust breeding in the Horn of Africa does not appear to have been on the scale expected. The reports made to the recent meeting of the Council of the East African Desert Locust Control Organisation in Arusha indicate that the threat of a major upsurge of *Schistocerca* is less imminent than at one time seemed likely.

There appear still to be some breeding swarms around the southern Red Sea and the Gulf of Aden, particularly on the coast of Somalia and in the tip of the Arabian Peninsula, and a few swarms have been observed crossing the frontier from Ethiopia into Sudan. This last country, however, is one of the best equipped and most efficient at dealing with this sort of threat, which seems to have been tackled promptly. Less certain is the situation in Ethiopia itself and in Eritrea, where under present conditions of intensifying military activity, locust surveying is extremely difficult, if not impossible. However, it has been reported that not only have both the Ethiopian, and the rebel Eritrean commands permitted the operation of some survey parties, but the Eritreans have also enabled a certain amount of control to be carried out.

Experts at the COPR in London reckon that most of the swarms in those areas that it has been possible to visit, have now been controlled, and there may in fact not have been much breeding in Eritrea itself. It is pointed

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out that this will have been winter breeding (which is what sparked off FAO's alarm earlier this year), and any resultant swarms, which would normally move out of this Eritrean breeding area, would by now have been reported from neighbouring countries. That no such reports have been received is a possible indication that the danger has receded, at least for the moment, and it is felt that the present pattern of breeding, as indicated by such reports as are available, is actually less widespread than that in January and March.

Despite this rather reassuring picture, there can still be no relaxation of control measures against such swarms and hopper bands as can be located, nor of vigilance over the whole desert locust area. Even in times of comparative recession—and it is 16 years since the last considerable outbreak—there are endemic solitary phase *Schistocerca* virtually the whole way across the northern half of Africa, and as far east as Afghanistan and Pakistan. Increasing swarms have been reported within the past two seasons in Libya, in the Yemen and in Saudi Arabia, and what are referred to as "low density scattered adults" in various areas under regular survey in Pakistan. This phrase may mean anything up to 1,000 insects per square kilometre, providing a nucleus from which breeding could cause a local outbreak rapidly if conditions are good and the first signs of swarming do not lead to rapid action.

The danger may lie in poor reporting leading to action coming too late. This is particularly so in the region where there is at present cause for concern, since it is here, in the central sector of the locusts' range and where with good

rains, three generations are possible in a year, that the risk of swarming is always the greatest. Both COPR in London, and FAO at its Rome headquarters, have noticed that whereas a few years ago locust officers in the field, and those responsible for surveyance in a number of countries, used to return complete report forms, filled in with every detail of importance, now—16 years after the big swarms—only casual reports are received, often incomplete and frankly inadequate. At a time such as the present, when there are known to be good conditions for the rapid breeding that leads to swarming, this is especially dangerous.

Much depends on weather conditions, which may be the factor deciding whether swarms in the Red Sea area migrate into central Saudi Arabia and thence on across the Gulf into Iran and Pakistan, or westwards into Sudan, across the Sahara and on to North Africa. That no one can survey all the area is evident: within the last few days, a message from the British Embassy in Sana'a brought a report of numerous small swarms in Eastern Yemen, where there has been no reported locust activity for many years—and this was not seen by locust control personnel, but apparently by a British official on some quite separate mission.

One thing which has made up for the poor quality of ground reporting is the increasing use of modern aerial remote sensing techniques. Infra-red photography from Landsat, combined with the data from meteorological satellites operating over the same regions, makes it possible to gain a very precise idea of the increase and type of vegetation in remote desert areas, in as small a parcel of land as 2 square kilometres. This can prove the most valuable early warning system available to those whose business it is to collect and collate material from all over the enormous area of potential locust breeding. The system is only in its infancy, but its possibilities are very great. In the words of a COPR locust officer, "there is no 'plague' at the moment. But the ingredients are there. What happens next depends on the weather, and on the efficacy of survey and control". Meanwhile, stocks of insecticides in some Red Sea countries, exhausted by the end of the winter breeding season, are being replenished through FAO's technical cooperation programme. \$800,000 have been allocated to Sudan, the Yemen and Somalia for this purpose and for renewal of equipment. Britain has provided the Desert Locust Control Organisation with two new spraying aircraft and has also virtually renewed the organisation's fleet of land rovers for ground operation.

Peter Collins



## Carter backs Third World technology

PRESIDENT Carter has proposed setting up a new foundation for technological co-operation to stimulate and support research of direct benefit to developing countries.

No firm decision has yet been made about the form that such a foundation would take. However according to Dr Frank Press, whose Office of Science and Technology Policy is looking at various alternatives, it could eventually achieve a stature comparable to that of the National Science Foundation, whose annual budget is now about \$700 million.

The proposal for a new foundation was first raised publicly in a speech which President Carter gave in Caracas during his recent visit to South America. Claiming that for the rest of the century the greatest potential for growth lay with the developing world, the president added that "to become more self-reliant, developing nations need to strengthen their technological capabilities".

The proposed foundation would concentrate primarily on developing new technologies, rather than on problems of economic development which are already the concern of other agencies. In addition, the administration emphasises that it would not merely attempt to develop research at home, but that much of the work financed by the foundation would be carried out abroad.

It is hoped that plans will advance sufficiently rapidly for the US to present the creation of the foundation to next year's United Nations conference on Science and Technology for Development.

Meanwhile the National Academy of Sciences has delivered to the State Department a report on the scientific and technological priorities which it feels should be addressed by the US delegation to the UNCSTD conference.

The report, which was prepared by a National Research Council committee chaired by Dr Press's immediate predecessor as presidential science adviser, Dr Guyford Stever, lists 22 unilateral and collaborative initiatives involving US research workers which it suggests should receive most emphasis.

These are grouped into five categories as follows:

● **Increasing food supplies:** Reducing post-harvest food losses; soil and water management at the farm level; plant and animal protection; and overcoming biological limits to plant productivity.

● **Health and related needs:** Delivery of primary health care; pure water and waste treatment; controlling infectious diseases in the tropics; and improved con-

traceptives.

● **Urbanisation and Industrialisation:** Improving urban settlements; planning transportation systems; building capabilities for creating and using industrial technology; and international research on the industrialisation process.

● **Management of resources:** Development and use of energy; remote sensing and other applications of satellite technology;

sustained, multiple use of forest resources; and research on the marine environment.

● **General initiatives:** Strengthening scientific and technological policymaking; information sharing; education in the United States; short-term, nondegree training; continuing university interchange and incentives for development-related research in the United States.

David Dickson

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*Come on in, the water's lovely—or is it?*

## UK wants collaboration on water pollution

CONCERN in the UK over EEC legislation to control water pollution has prompted the UK Water Research Centre (WRC) to set up a European Division. It is to help bridge the gap on water pollution policy between the UK and the other countries of Europe.

At six-weeks-old, the division is too young to know how it will tackle its task. But Dr T. V. Arden, its director, sees the first priority as making contact with most of the water centres and authorities in Europe to find out what research is being done where. At the moment he feels there is too much overlap of research effort and too little pooling of research results.

Once contact is made pollution scientists should be able to discuss their countries' problems before approaching the EEC with recommendations for action. At present, Dr Arden says, EEC legislation can be initiated by one or two people working on a specific problem relevant to their country. Working through the bureaucratic machinery of the EEC, their recommendations can become legislation having received no substantial input from the scientists of another country. Poorly thought-out policies, which can even be irrelevant to some countries' problems, can be introduced in this way, Dr Arden believes.

A case in point is the EEC directive on the quality of coastal bathing waters. It lays down standards for the concentrations of bacteria in sea water and recommends several methods of measuring them. The directive is particularly inappropriate to the UK, according to Dr Arden. Britain's long coastline would make continual monitoring of bathing waters prohibitively expensive and the heavy seas some-

times found around Britain would probably pose more of a hazard to the people taking samples than any bacteria would pose to bathers.

The WRC also questions the validity of measuring bacterial levels at all. The UK Medical Research Council (MRC) sponsored a study some years ago to assess the hazards to health from bacteria in coastal waters. It found that provided the waters are aesthetically acceptable the danger to health is minimal. The study also found that sewage outfall is not the only factor contributing to high bacterial levels: seagulls can also have a marked effect on them.

Work at the WRC has also shown that the concentration of bacteria measured varies with each method recommended by the directive to a far greater extent than generally realised. Many of the methods of counting bacteria already developed apply to potable water and may not be suitable for sea water. One of the centre's current programmes, therefore, is to assess the comparability of the results obtained by the various methods known to be in use.

Dr Arden is confident that the WRC is the best centre of its kind in Europe and feels that it is therefore natural that it should take the lead in increasing collaboration. Some of the other centres excel in specific research topics and he is especially keen to make contact with these. Eventually, he would like to collaborate with some of the international agencies such as the United Nations Environment Programme, but he feels that it will be six months before the division really gets off the ground.

Judy Redfearn



## European Molecular Biology

**Peter Newmark** attends the opening of a new cooperative research venture

THE European Molecular Biology Laboratory (EMBL) in Heidelberg has become an official fact. Dr V. Hauff, the West German Minister for Research and Technology, inaugurated the laboratory on 5 May. Sir John Kendrew, Director-General of EMBL, said he was delighted that at last the laboratory was "no longer just an item on the agenda of some boring inter-governmental meeting."

EMBL has one central laboratory, ten minutes drive from Heidelberg town, and two additional outstations, one at DESY in Hamburg, and the other at the Institut Laue-Langevin in Grenoble. The central laboratory will eventually contain about 90 scientific staff (including engineers) but not all of them have yet been appointed. There is also a considerable number of "fellows"—mainly post-docs who bring their own funds—and visiting scientists. The laboratory is loosely split into three divisions: cell biology, biological structures and instrumentation. Only in the latter is there a substantial number of positions still to be filled. What little room is left elsewhere is strongly rumoured to be reserved for the arrival during 1979 of Professor K. and Dr N. Murray on a three-year secondment from Edinburgh University and of Professor J.-E. Edstrom from the Karolinska Institute.

The seeds of EMBL (known by its inhabitants as ee-em-be-el) were sown in 1962—not by biologists but by physicists. Both Sir John Kendrew and Professor E. C. Slater (President of the EMBL Council) paid tribute to Victor Weisskopf, then Director of CERN and a Viennese American, and the late Leo Szilard—a Hungarian American—who between them hatched the idea that European biologists should have a counterpart to the physicist's CERN. Their idea was received with enthusiasm by European molecular biologists, including Sir John, who were concerned at the "brain drain" to the US, and by those committed to greater European collaborations. In 1963 a

group of interested scientists formed the European Molecular Biology Organisation (EMBO) to further their aims which included not only a laboratory, but also fellowships, training courses and research grants. Only the last remains to be achieved.

For two years from 1963 EMBO pursued its hopes with, according to Professor Michael Sela head of the Weizmann Institute, their stationery and stamps paid for by Israel. Israel has ever since counted as being in the "European Area", at least when it comes to molecular biology. In 1965 the Volkswagen Foundation provided the first substantial funding that allowed EMBO to go into action with fellowships and training. By 1970 a proper intergovernmental organisation, the European Molecular Biology Conference (EMBC) had been formed by the governments of 13 countries which proceeded to fund EMBO and to consider the setting up of EMBL. This began its formal existence in 1974. Sir John Kendrew was appointed in 1975 and the laboratory is now supported financially by ten of the member states of EMBC.

The EMBL is now governed by a Laboratory Council and EMBC no longer has any legal responsibility for EMBL. The joint secretariat of both EMBC and EMBO (which is also a separate entity with its own funds) has its offices in the EMBL at Heidelberg.

With its ancestry in CERN it is not surprising that EMBL has adopted its conditions of appointment and advancement from its senior relative. That means that initial appointments are for a maximum of three years, with the possibility of renewal for a maximum of nine years or of tenure at any stage. So far a few people have reached the first three-year renewal period, not all successfully, but no one has yet been given tenure. "It is something that I shall restrict and resist," Sir John Kendrew told *Nature*. Sir John wishes to maintain the flexibility which, he feels, is so scarce elsewhere in

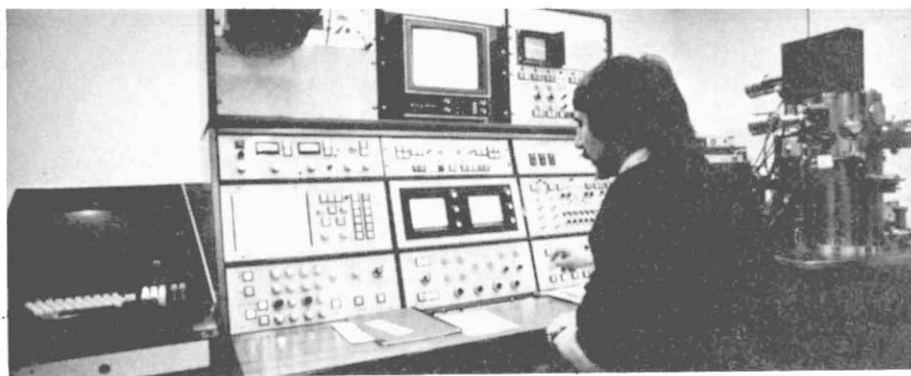


*Above: the laboratory and its main entrance, spelling out European cooperation. Top right: EMBL's scanning transmission electron microscope in action. Right: West Germany's Dr Hauff at the opening ceremony and (far right) in conversation with the director-general Sir John Kendrew*

Europe. He would, however, be prepared to grant tenure to any scientist of "senior status" but none has yet been appointed—except for Professor Leo de Maeyer, who has just taken up the position of Head of the Division of Instrumentation. But even this appointment is limited to a three-year secondment from the University of Louvain.

Professor de Maeyer's arrival nevertheless heralds the real effort to establish the central feature of EMBL. From the beginning it was appreciated that EMBL should offer, wherever possible, facilities that would exceed the capabilities of any member nation. And it was in instrumentation that that aim was seen to be most achievable. Paradoxically this central feature is the last to get going since it is only with the completion of the central laboratory that the necessary space and stability has been found. For the present, instrumentation centres on the development of a scanning transmission electron microscope, computers and computer graphics, and synchrotron radiation monitors for the Hamburg outstation. The expansion of the instrumentation division will probably focus on the development of cell sorters, other microanalytical methods, and X-ray detectors. Sir John Kendrew told *Nature* that he intended to establish a committee that would help determine the instrumentation needs of member





## China encourages school science

DEVELOPMENTS in China continue to reflect the new emphasis on education and science. At the end of April, a month after the science conference, the Chinese Ministry of Education organised a national conference on educational work. In the opening speech, Teng Hsiao-ping repeated Chairman Hua's call to train a mammoth contingent of working class intellectuals and to raise the scientific and cultural levels of the entire nation.

Attention was not to be confined to higher education, Teng said. The scientific content of both primary and secondary school curricula was to be increased. Furthermore, differences in academic ability were no longer to be covered up. Special training of the talented could be combined with broad educational opportunities and students treated differently, so long as the aim remained the creation of conditions for every individual to advance and not the suppression of some for the benefit of others.

Education as a whole was to keep pace with the needs of agriculture and industry, and state departments would work together to integrate the educational programme into the framework of the national economy. Finally, Teng said, the country's nine million teachers held the key to success. They should be respected by their pupils, while they themselves should continue to improve themselves politically and professionally, making full use of post-experience courses, new reference materials, modern teaching aids, radio and television.

Another significant development has been in the *Kwangming Daily*, one of the three national newspapers published in Peking, which since 1 May has devoted most of its issues to scientific and educational coverage.

And from the province of Anhwei comes the news that, of the 700 new students starting at the Chinese Scientific and Technological University, 92 are under 16. They are students who, with the help of friends and teachers, have progressed well beyond formal levels of education through self-study.

**T. B. Tang**

## Core curriculum

IN a decision likely to have implications for university courses across the USA, the faculty of Harvard University voted last week to accept a new "core curriculum"—including a course in scientific methodology and an obligatory test of mathematical competence—to be taken by all undergraduates.



nations. He said it was also possible that instruments would be developed for commercial exploitation with royalties going to EMBL.

The other major feature of EMBL which may never be matched in Europe is its high containment recombinant DNA facility. This was added to the plans of EMBL in 1976 and is now nearing completion. It occupies a separate building of 700 m<sup>2</sup>, which consists of a series of normal open laboratories and offices separated from a series of high containment laboratories by a range of showers and autoclaves through which all personnel and equipment have to pass. The high containment facilities are not expected to become operational before the end of 1978, in part because the German government has only just adopted its guidelines for recombinant DNA research. In the meantime staff are being specially trained and a committee is being established to assess priorities for use of the laboratories by visiting and staff scientists. Most of the latter have recently taken up residence at EMBL.

Still under discussion is the possibility of setting up courses in training in recombinant DNA techniques, and the question of the extent to which visiting scientists will be allowed to carry out their own experiments rather than entrust them to EMBL technicians. Despite some local antagonism to the laboratory ("The Monster in the City Woods", according to a January issue of the student paper *Heidelberger Rundschau*), it is anticipated that P4 experiments will be smoothly under way within a year.

The EMBL does not have its own lecture theatre suitable for gatherings

as large as the 200 people who attended the inauguration for which it made use of the facilities of the Max Planck Institute für Kernphysik (nuclear physics) next door. It does, however, have an ample canteen and a large library. It did not escape my attention that the longest complete run of a journal on display in the library was that of *Nature*. It seemed very fitting for the European Molecular Biology Laboratory that their *Nature* started with that 1953 volume which contained Watson and Crick's "A Structure For Deoxyribose Nucleic Acid."

The inauguration ceremony was attended by some 200 dignitaries including Dr Hauff, the Ministers of Research of Austria, France and Sweden and many eminent scientists who have acted or still serve as consultants, advisers or members of the Laboratory Council. Dr Hauff, who was a last minute replacement for Dr W. Scheel, President of West Germany, detained by the state visit of Mr Brezhnev, used the occasion to deliver a remarkably strong statement in support of the freedom of scientific investigation. Science, he said, was an integral part of the spiritual and intellectual freedom that was essential for the survival of a democratic state. The state should not pander to that group of sceptics among the educated strata who wished the state to intervene in the course of scientific investigation. Only when that course departed from the intellectual search for scientific truth to impose itself upon society did Dr Hauff believe that the state should consider setting limits. As an example of that he cited research on recombinant DNA.





# correspondence

## An empirical test of Parkinson's Law

SIR,—Parkinson<sup>1</sup> framed the law that Work Expands To Fit The Time Available For Its Completion and showed one consequence of this to be that the number of administrators within a public organisation tends to increase irrespective of the amount of external work done by that organisation. This is partly because administrators spend much of their time sending minutes to each other. It follows that bigger organisations should have a higher proportion of administrators, because each one has more colleagues with whom to communicate and therefore needs more assistants. This prediction is verified by the figure, using data taken from the Natural Environment Research Council's report for 1976–77 (HMSO).

The function of NERC is to do scientific research on the natural environment and it is divided into several component bodies (see table). Although the bigger bodies do have relatively more 'non-scientific staff' than smaller ones (for the figure the correlation coefficient  $r=0.64$ , the probability that this relationship occurred by chance  $P<0.05$ ), three bodies (ITE, IOS, IGS) are unexpectedly efficient (defining efficiency as  $(1-A)/A$ , where  $A$  is the proportion of non-scientific staff). The three efficient organisations are decentralised, each being dispersed amongst several different addresses. It seems that the proportion of non-scientific staff within NERC bodies is determined largely by the total staff employed ( $S$ ) and the number of addresses amongst which it is dispersed

( $D$ ) according to the regression equation  $A=22.23 \log S-1.86D-8.77$

Eighty-one percent of the variation in  $A$  is accounted for by variations in  $S$  and  $D$ , and both regression coefficients are statistically significant ( $P<0.001$  and  $P<0.01$  respectively). We may conclude that big, centralised bodies have proportionately more supporting staff and fewer scientists than small or dispersed organisations.

It will be noted that it is the logarithm of  $S$  that is used in the above equation. This is because the proportion of supporting staff in NERC bodies increases most quickly in small organisations, and levels off near 50% when the total staff exceeds 120. This is a ratio of one non-scientist to one scientist and it may be difficult to increase this ratio whilst continuing to do scientific research. This may be Parkinson's 'maximum ratio'.

What action should be taken as a result of this empirical confirmation of Parkinson's Law? It depends on your viewpoint. If you are an administrator in a scientific organisation, you might aim for small research units dispersed at several addresses to maximise efficiency. If you are a scientist in a research organisation, you might aim to work in a large organisation (over 120 total staff) to maximise the amount of administrative help available to you.

We can be confident that Parkinson's Law is of general application: the number of administrators in an organisation expands irresistibly in response to the first law of sociodynamics. However, the equation above was determined for NERC, and we cannot

assume that it will be applicable in exactly the same form to other types of organisation. The constants may differ. The 'maximum ratio'  $A/(1-A)$  may differ as may the value of  $S$  at which this ratio is achieved. Furthermore, 20% of the variation in  $A$  remains unexplained.

I thank Professor C. J. Krebs, Dr A. Watson, Dr S. P. Dempster, J. G. Ferguson and Mary Stevenson for useful discussion and criticism.

R. Moss

*Institute of Terrestrial Ecology,  
Banchory,  
Kincardineshire, Scotland*

*1. Parkinson, C. N. Parkinson's Law. London, John Murray (1958).*

## All research is collaborative

SIR,—As an observer and to some extent a promoter of collaborative research projects between one developing country and, I hope, another (Egypt and Britain) I was mystified by the breathless tone of Robert Walgate's recent article (and its postscript) on research in third world countries (2 March, page 8). There is surely nothing surprising, unusual or peculiar to the third world about the situation he describes.

Almost all science is collaborative wherever it is done. At the very least it is funded by one organisation with its own set of priorities and done by individual scientists with unique personal objectives. Collaborators, both individuals and institutions, often disguise their true motives in order to get what they want, or more of what they want, from the other party. Give and take is, and has to be, the order of the day because it seems to be the way we are made to work. We have all both exploited and been exploited from time to time.

Issues are naturally more sensitive when the parties to a collaboration have very different material resources to command. Few things require as much mature self-confidence as the easy acceptance of gifts which cannot be repaid, particularly when the donors (as sometimes) seem to feel that eternal gratitude and negligible criticism are their proper due. Some scientists from wealthy institutes collaborate with those less well off with genuine philanthropy and impeccably idealistic motives. Others (both individuals and institutions) are more selfish, hoping to benefit themselves. I do not believe that many scientists in developing countries doubt this or are surprised by it. They accept (or reject) offers of collaborative help with their eyes open, recognising that work in which all have something to gain is likely to be most fruitful. Dr Nayudamma's comments sound so sensible and ring so true that we would do well to accept them as all the guidelines that are necessary.

Was Robert Walgate's tongue in his cheek when he typed his final quotation from Dr Oldham? Surely the last thing any of us (developed or not) need is a collaborative research project on collaborative research.

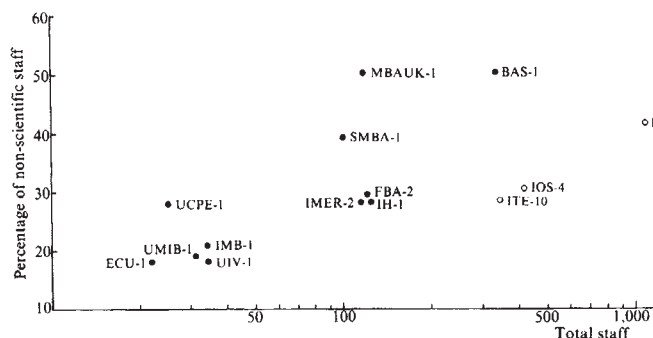
GEOFFREY R. PENZER

*British Council, Cairo, Egypt*

Numbers of staff in component bodies of NERC (1976-7 report)

| Name                                        | Initials<br>(see figure) | Total<br>staff  | Scientific<br>staff | Number of<br>addresses |
|---------------------------------------------|--------------------------|-----------------|---------------------|------------------------|
| Experimental Cartography Unit               | ECU                      | 22              | 18                  | 1                      |
| Institute of Geological Sciences            | IGS                      | 1101            | 652                 | 12                     |
| Institute of Hydrology                      | IH                       | 123             | 88                  | 1                      |
| Institute of Marine Biochemistry            | IMB                      | 34              | 27                  | 1                      |
| Institute for Marine Environmental Research | IMER                     | 114             | 82                  | 2                      |
| Institute of Oceanographic Sciences         | IOS                      | 417             | 290                 | 4                      |
| Institute of Terrestrial Ecology            | ITE                      | 345             | 250                 | 10                     |
| British Antarctic Survey                    | BAS                      | 337             | 170                 | 1                      |
| Unit of Invertebrate Virology               | UIV                      | 34              | 28                  | 1                      |
| Freshwater Biological Association           | FBA                      | 120             | 85                  | 2                      |
| Marine Biological Association of UK         | MBAUK                    | 119             | 60                  | 1                      |
| Scottish Marine Biological Association      | SMBA                     | 100             | 61                  | 1                      |
| Unit of Comparative Plant Ecology           | UCPE                     | 25 <sup>a</sup> | 18 <sup>a</sup>     | 1                      |
| Unit of Marine Invertebrate Biology         | UMIB                     | 31 <sup>b</sup> | 24 <sup>b</sup>     | 1                      |

<sup>a</sup>Including 11 post-graduate students <sup>b</sup>Including 15 post-graduate students (December 1977).



The percentage of 'non-scientific staff' and the total number of staff in component bodies of NERC. The black spots are centralised bodies with 1 or 2 addresses, the open circles are decentralised bodies with 4-12 addresses.

# news and views

## The Tasmanians: the longest isolation, the simplest technology

from Jared M. Diamond

WITH an area of 26,000 square miles and a cold maritime climate like that of Britain and Tierra del Fuego, the island of Tasmania lies 150 miles from the coast of southeast Australia. When Europeans first explored Tasmania towards the end of the 18th century, they found it inhabited by 4,000 dark-skinned, frizzly-haired, stone-age people related to the Australian Aborigines. The distribution of these people was already a source of astonishment to Matthew Flinders, the navigator who in 1798 proved Tasmania to be an island separate from Australia. In the Bass Straits between Tasmania and Australia are some moderate-sized islands that were uninhabited at the time of their discovery by Europeans despite being wooded, well-watered, and supporting game animals: King Island, of 420 square miles, 40 miles from Tasmania, and the Furneaux Group, of 770 square miles, 14 miles from Tasmania. The native Tasmanians had bark rafts with which they regularly visited islets within 5 miles of Tasmania to hunt seals and muttonbirds, and which could probably at least occasionally succeed in crossing water gaps of 6–14 miles, but which became waterlogged and sank within a day or two. How did these people reach Tasmania from Australia, across water gaps far exceeding the range of their watercraft? In reaching Tasmania, how could they have failed to reach or survive on King Island and the Furneaux Group en route? Having reached Tasmania, how could they then have failed for unknown millenia to colonise the Furneaux Group, which is visible from Tasmania and at least marginally within range of their craft?

New understanding of these problems is synthesised in recent articles by Rhys Jones of the Australian National University, who has made major contributions to Tasmanian archaeology and cultural anthropology (*Quaternary*

*Studies* (eds Suggate & Cresswell) 21 (Royal Soc. New Zealand, Wellington, 1975); *Problems in Economic and Social Archaeology* (eds Sieveking, Longworth & Wilson) 235 (Duckworth, London 1976); *J. hum. Evol.* 6, 353, 1977; *Sunda and Sahul* (eds Allen, Golson & Jones) 317 (Academic Press, London); *Stone Tools as Cultural Markers: Change, Evolution, and Complexity* (ed. Wright) 189 (Australian Institute of Aboriginal Studies, Canberra 1978); *Explorations in Ethnoarchaeology* (ed. Gould) (University of New Mexico Press, Albuquerque 1978)). Jones shows that this biogeographic puzzle was also a cultural experiment in isolation unique in recent human history.

The Tasmanians had the simplest material culture of any modern humans. Their stone tools were few, simple, and unhafted; they lacked agriculture, dogs and other domesticated animals, bone tools, bows and arrows, and boomerangs; they could not light a new fire, and had to carry smouldering sticks to perpetuate fire; they endured Tasmania's cold climate with no dwelling except simple windbreaks, and no clothes except rough capes of wallaby skins; and their main food-gathering tools were wooden digging sticks and spears. Within a few decades they were nearly exterminated by European settlers, until the scattered survivors were removed in 1834 to Flinders Island, where the last full-blooded Tasmanian died in 1876.

Besides these tragic humans, Tasmania supported other native mammals: the platypus and echidna, as well as numerous marsupials such as kangaroos, wombats, possums, bandicoots, and the Tasmanian Wolf and Devil. No one doubts that these other mammals walked from Australia rather than swam or paddled; and Jones argues convincingly that humans likewise arrived on foot. The archaeological record proves that Pleistocene humans reached Australia from Wallacea at least 40,000 years ago, and Tasmania

from Australia at least 22,000 years ago (Jones *Nature* 246, 278; 1973). At that time, sea-level was about 300 feet below its present stand because so much of the Earth's water was locked in glaciers. What is now the shallow Bass Straits was then a broad land-bridge joining Tasmania to Australia. Over this bridge walked humans and kangaroos, just as rhinoceros and elephants walked from Asia to what are now the islands of Sumatra and Borneo. When the sea-level rose rapidly at the end of the Pleistocene 12,000 years ago, most of the land bridge was drowned, and Tasmania, King, and the Furneaux groups were cut off as islands, leaving humans stranded along with other mammals. A few stone tools testify to the former presence of humans on King and Furneaux.

Why did the stranded humans survive on Tasmania but not on King or Furneaux? Because, Jones reasons, the latter two islands were insufficiently large to support more than around 200 or 500 humans, respectively, and such human populations are too small to survive long in complete isolation. The same fate befell the Pleistocene human population isolated on Kangaroo Island, 8 miles from the coast of south Australia. These populations died for lack of people, not for lack of food. Jones's analysis makes a bridge from anthropology to biogeography, for biogeographers have emphasised that minimum stable population sizes explain striking species differences in critical area requirements and hence in ability to survive on isolated islands (for example, Darlington, *Zoogeography* (New York, Wiley, New York, 1957); Brown *Am. Nat.* 105, 467; 1971; Wilson & Willis in *Ecology and Evolution of Communities* (eds Cody & Diamond) 522 (Harvard University Press, Cambridge, 1975)). The elegant analysis by Jeanette Hope at the Australian National University of the fossil record (*Proc. Roy. Soc. Vic.* 85 (2), 163; 1973) proves that populations of

Jared Diamond is Professor of Physiology at the University of California Medical Center, Los Angeles.



the marsupials and monotremes stranded with humans on King, Furneaux, and Tasmania similarly suffered differential extinction related to island area.

Once cut off by rising seas at some time between 12,000 and 8,000 years ago, the Tasmanians became the most isolated, closed society in the world, with no outside contact until European explorers arrived—as Jones expresses it, “the world’s longest isolation, the world’s simplest technology”. Why was their technology so simple? Part of the answer is that it was already simple at the time they were cut off. The Tasmanians arrived with a characteristic core-tool and scraper stone-age technology that was widespread over Australia until 5,000 years ago, when a new culture with the dingo and more diverse stone tools reached Australia, presumably from Wallacea. By then, Tasmania was isolated from the new culture by Bass Straits. But, astonishingly, the other part of the answer is that the Tasmanians’ technology became simpler during the thousands of years of isolation. The most striking examples are the loss of the art of fishing and of making bone tools. The archaeological record shows a reduction in diversity of bone tools in the millennia after 8,000 BP, until both bone tools and fish disappeared from middens around 3,500 BP. Yet the same fish species that were formerly a main component of the Tasmanian diet, and the

mammal species used as sources of bone tools, were still available and common when Europeans arrived. These cultural losses were economically senseless and could occur or persist only in a completely isolated people, cut off from reintroduction of these technologies. It remains unclear whether edge-ground axes, hafting, boomerangs, and barbed spears were also initially present and subsequently lost, or whether these arts never reached Tasmania in the first place. For reasons that afford an interesting subject of speculation, bark rafts were absent from the part of Tasmania opposite the ‘attractive menace’ of the visible Furneaux islands, but persisted elsewhere on the Tasmanian coast.

Jones’s solution of the Tasmanians’ biogeographic puzzle should encourage anthropologists and archaeologists to address other equally important issues. What can one learn about convergent cultural evolution from the numerous, independently derived economic parallels between the native peoples of Tasmania, south New Zealand, and Tierra del Fuego, long divergent in ancestry but living in similar habitats and climates? Why were native human population densities as high on Tasmania as on the adjacent Australian mainland, despite the much simpler technology of the Tasmanians? And why did technology regress in the tiny Tasmanian universe during its millennia of isolation? □

such early Precambrian deposits as the Huronian of Canada or the Witwatersrand succession of South Africa, both more than 2,000 million years in age. Within the last year Fyfe, (*Nature* 267, 510; 1977) has supported the Hargraves model and Windley, (*Nature* 270, 426; 1977) and Moorbath, (*Nature* 273, 75; 1978), have criticised it, quoting geological observations which seem to be inconsistent with the hypothesis. When authorities disagree one can suspect that an interesting explanation may be round the corner which may reconcile parts of the thinking of both camps. In fact the difference of opinion centres largely on the timing of the emergence of continental crust, a critical point in Hargraves’s thesis. There is general agreement that the Earth has passed from a period of widespread crustal instability in Archaean times through stages when greater parts of the crust were stabilised to reach the large plates of the present day. There is also wide recognition of the extent of submarine volcanicity when the Archaean greenstone belts were forming. But the hypothesis that high heat flow limited the thickness of the crust and so prevented the early emergence of land appears to be contradicted by Archaean crust reported as 30 to 50 kilometres thick. This seems to be the essential issue in the debate.

The work of Pettijohn and Canadian colleagues on the Huronian of Canada and of Simonen and the Finnish and USSR Geological Surveys on the Precambrian sediments in the eastern Baltic Shield revealed shallow water sediments laid down on the margin of Archaean blocks. With the radiometric age determinations available in the 1960s these Archaean masses could be identified as two of the earliest continents in the sense that they formed long lived land masses probably established between 2,500 and 2,000 million years ago. About 40 blocks of Archaean rocks are known; together they make up about 5% of the continental crust. The largest, in eastern Canada is 2,000 kilometres across, most are much smaller. Shallow water deposits ringing some, but by no means all, of these masses, suggest that they formed islands. If so, their area would have been comparable to that of today’s continental shelves implying that 2,000 million years ago at least as much continental crust was above water as is today submerged on continental margins. But much of the remainder of the continental crust at that time was accumulating marine sediments so that the Proterozoic continental shelves may have been remarkably extensive. Because of later deformation and metamorphism it is difficult to establish either the extent

## Timetabling the emergence of continents

from John Sutton

THE extent to which the sea has spread over the land has varied greatly and has interested mankind at least since Biblical times. In the late Cretaceous, 80 million years ago, the continental shelves were six times as extensive as at the present day, and every State of the Union contains deposits left by the Cambrian Seas which flooded North America 500 million years ago. But, even at the greatest advances of the past 600 million years probably as much as two-thirds of the continental crust remained as dry land. However, matters may not always have been so.

The primitive Earth may have had a completely submerged outer shell and the continental crust, as we know it today, would, if spread as a continuous shell, underlie the sea as a layer 12-15

kilometres thick. No crust survives from the earliest 800 million years of the Earth’s history, but portions of crust from 3,700 million years onwards are preserved. The oldest surviving rocks show evidence both of submersion and of erosion and thus by implication of emergence at least to sea level.

The extent to which the Precambrian crust stood above sea level is currently a matter of debate and in a recent issue of *Nature* (273, 22; 1978) Michael LaBarbera suggests that the first appearances of the Metazoa late in the Precambrian might be linked to the general emergence of the continents at that time, which Hargraves, (*Science*, 193, 363; 1976), has proposed. As Hargraves himself pointed out in his proposal, such a model of the Earth, which places the first general emergence of the continents between 1,400 and 1,000 million years ago, does not readily explain

John Sutton is Professor of Geology at Imperial College, London

or depth of such Proterozoic shelves.

Going further back in time, all are agreed that Archaean volcanic piles reached the ocean surface and suffered erosion. But as Moorbath and Windley point out, (as indeed does Hargraves), granitic crust was exposed and eroded during the Archaean. In West Greenland and the South of Rhodesia, virtually the only areas where rocks over 3,500 million years old have been mapped in detail, evidence of erosion has been found. Accordingly, there must have been some land as far back as 3,700 million years ago, though the areas of rocks as old as this are too small to indicate how extensive such land may have been.

The very much larger areas of rock over 2,500 million years old, in all cover several million square kilometres scattered across all the continents. Everywhere the cover rocks dating from about 3,500 – 2,500 million years tell much the same story. There was abundant submarine vulcanism, its underwater origin indicated by pillow lavas chilled in water, within the Archaean greenstone belts. As Fyfe has pointed out the associated granites, (strictly speaking tonalites), were probably also formed below the sea, a situation which has no present day analogue. Shallow water deposits, some with fossil blue-green algae and stromatolites requiring illuminated shallow seas, are found with the volcanics, some of which rest on eroded granite crust as at Belingwe in Rhodesia. Erosion of such rocks was required to produce the Witwatersrand succession over 2,300 million years old in South Africa where the gold and uranium was derived from older crust.

The flat lying, and at least in part, shallow water deposits of the Witwatersrand are the earliest example of sediments laid down by advance of the sea over a stabilised granitic crust. From that time onward successively larger stable areas developed, principally after two peaks of gneous activity at 1,900 – 1,700 and 1,200 million years ago. There seems to be a relationship between the periodic additions of igneous rocks to the crust and subsequent increased stabilisation, for after each such addition larger stabilised land masses stood above sea level. Erosion from these contributed to the extensive late Precambrian deposits noted by Hargraves.

The scenario described above suggests that granitic crust has emerged above sea level periodically since at least 3,700 million years ago, but, as with Hargraves's model, emphasises that much continental crust was submerged early in the Earth's history. The earliest permanent land masses may have been those formed in the Proterozoic between 2,500 and 2,000

million years ago.

In addition to the Archaean assemblages of volcanic and granitic rocks, there are a number of early Precambrian high grade metamorphic terrains which contain metamorphic assemblages suggesting formation at depths of 30 to 50 kilometres. Seismic investigations indicate similar thickness of crust below Precambrian shields and reconstruction of the crust below Colorado based on inclusions in kimberlites which have penetrated the outermost 150 kilometres of the Earth suggest a thickness of the same order. Where one can see rocks suspected as coming from the lower crust they are a mixed assortment of granulitic rocks denser than granite but with densities in the range 2.7 – 3 and thus lighter than the Upper Mantle.

Hargraves's assumed Precambrian high heat flows limited the continental crust to the thickness beyond which granite starts to melt. The top of the resulting thin crust remained below sea level until falling temperatures in the late Precambrian allowed thickening and emergence. What may

in fact have happened is that the depth of granitic rocks was restricted just as Hargraves has indicated but that below an upper granitic crust a granite free granulitic lower crust, perhaps twice as thick, formed, so providing the 30 or 50-km thick Precambrian crust which the geological record suggests. As Fyfe himself has indicated, granulites could form in precisely this way where continental crust is taken below the depths at which granites start to melt.

It would be of interest to repeat Hargraves's calculations allowing both for a possible granulitic lower crust beneath a limited thickness of granite and for the subsequent later additions to the crust discussed by Moorbath and Windley. Such a model might permit widespread early submergence of continental crust with the irregular emergences of land from Archaean times onward which the geological record appears to require. Shallow water habitats in this timetable would have been present throughout the geological record.

□

## Complete sequence and secondary structure of a viroid

from Roger Hull

VIROIDS are pathogens of higher plants and so far have been implicated as the causal agents of about seven diseases. They seem to exist only as self-replicating uncoated ribonucleic acid molecules with many unusual properties which differentiate them from the RNAs of viruses. Much of the work on the structure of viroid RNA is done by two groups, those of H. L. Sanger at Giessen, West Germany and of T. O. Diener at Beltsville in the US. They have found that viroids are unique in their small size (molecular weight about 120,000) and their single-stranded circular structure (see *News and Views* **265**, 497; 1977; McClements & Kaesberg *Virology* **76**, 477; 1977). Cutting of the circular structure results in loss of infectivity (Owens *et al. Proc. natn. Acad. Sci. U.S.A.* **14**, 3859; 1977). It was only a matter of time before the sequence of the nucleotides that make up a viroid was determined. In this issue of *Nature* (Gross *et al.*, page 203) is reported the collaborative work between Sanger and a group in Munich, which has resulted in the elucidation of the complete sequence of nucleotides in potato spindle tuber viroid (PSTV) and the prediction of

its secondary structure. A companion paper, giving more details of the sequencing techniques has recently been published (Domdey *et al. Nucleic Acids Res.* **5**, 1221; 1978).

Sanger and his coworkers were unable to use *in vivo* labelled nucleic acid because of its low specific activity. Therefore, after highly purifying PSTV RNA, they labelled the 5' nucleotides of oligonucleotides produced by nuclease digestion and used recently-developed RNA sequencing techniques (Donis-Keller *et al. Nucleic Acids Res.* **4**, 2527; 1977; Simonits *et al. Nature* **269**, 833; 1977). They sequenced the RNase T<sub>1</sub> and RNase A oligonucleotides and then put these in order using overlapping fragments obtained by controlled digestion by these and other enzymes. The oligonucleotide catalogue showed that PSTV RNA comprised 359 nucleotides giving a molecular weight of 115,480 which is in good agreement with the estimate of 119,500 obtained by biophysical techniques (Sanger *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 3852; 1976) and corrected for Mg<sup>2+</sup> and Na<sup>+</sup>. The nucleotide sequence confirmed the circular nature of the viroid molecule.

It has been shown previously that viroids do not function as messenger RNAs in *in vitro* translation systems

Roger Hull is at the John Innes Institute, Norwich.



(Davies *et al. Virology* **61**, 281; 1974; Conjero & Semancik *Virology* **77**, 221; 1977; Semancik *et al. Virology* **80**, 218; 1977) nor do they interfere with the translation of known messenger RNAs (Hall *et al.* **61**, 486; 1974; *Virology* **80**, 218; 1977). The primary sequence of PSTV RNA shows that the common AUG initiation site is missing though there are seven of the more unusual GUG initiation sites and six possible termination sites. The authors point out that it is difficult to envisage ribosome binding sites at the 5' side of the GUG initiation triplets and that there is no 'cap' structure present. Thus the evidence from the primary sequence supports the idea that PSTV RNA lacks messenger activity.

Having obtained the primary sequence of the molecule Sanger and his colleagues predict its most probable secondary structure. They first arranged the structure to give the maximum number of base pairs. They then modified this model to take account of the location of nuclease cleavage sites found in controlled digests and the sensitivity of cytidines to bisulphite. In the companion paper (*op. cit.*) the experiments investigating the sensitivity of cytosines to modification to uridine by bisulphite are described, this modification only occurring in single-stranded loops, not in double-stranded regions. The model that they derive for the secondary structure of PSTV RNA is of a series of double helical sections with short single-stranded loops in between and at each end. They predict 122 base pairs which would mean that 68% of the nucleotides are base paired. This model is in agreement with that suggested from thermodynamic and kinetic studies (Henco *et al. Nucleic Acids Res.* **4**, 177; 1977) and gives the rod-like shape that is found when undenatured viroid RNA is examined under the electron microscope (Gogo *et al. Virology* **55**, 70; 1973).

The knowledge of the primary sequence and secondary structure of viroid RNA helps little however, in understanding the key question of how viroids work. One point from the sequence which may be of significance is the occurrence of a long sequence of purines, mainly adenosine, a feature which is also found in other viroids (quoted in this *Nature* paper). When other viroid RNAs are sequenced it will be interesting to see what other features they have in common. However, I do not think that the answer to the question of how viroids function will come from sequences and structure. It is much more likely to be found from studies on their *in vitro* and *in vivo* replication. Such work is already in progress with reports of the *in vitro* synthesis of RNA complementary to PSTV (Owens & Diener *Virology* **79**,

109; 1977) and of *in vivo* studies in protoplasts (Mühlbach & Sanger *J. gen. Virol.* **35**, 377; 1977; Mühlbach *et al. Plant Sci. Lett.* **8**, 183; 1977) and in whole plants (Conjero & Semancik *Virology* **77**, 221; 1977). □

## Laser-switched collisions

from Peter Knight

THE nonlinear optical processes by which an atom can be excited by simultaneous collisional interaction whilst absorbing or emitting photons in a laser field have been examined by several theorists recently. The simplest example of such a 'laser-switched collision' was discussed by Gudzenko and Yakovlenko (*Sov. Phys. JETP* **35**, 877; 1972) and by Harris and Lidow (*Phys. Rev. Lett.* **33**, 674; 1974) and is illustrated in Fig. 1.

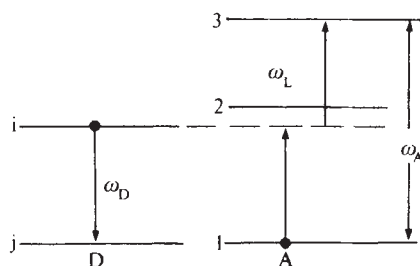
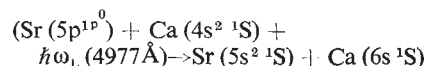


Fig. 1 Dipole-dipole transfer example of a laser switched or radiative collision.

Atom A is excited to its second excited state by collisional energy transfer from atom D a distance  $R$  away simultaneous with photon adsorption from a laser field at some frequency  $\omega_L$ . The energy transfer process is assumed by way of a dipole-dipole interaction, with  $R$  a slowly varying (compared with other characteristic times) parameter and where the atoms are assumed to be far enough apart for a product wavefunction representation to be adequate. A novel feature of the process is that the resonant laser frequency does not match a transition frequency in either donor D or acceptor A but the difference  $(\omega_A - \omega_D)$ . The mixture of A and D absorbs preferentially in a region where component elements do not absorb. The collisional excitation of A from state 1 to state 3 by a passing atom D in state i without a laser field is vanishingly improbable if the energy defect  $(\omega_A - \omega_D) \gg kT$ ; if A can

absorb a photon to complete the transition from the virtual, energy non-conserving intermediate state to a real energy conserving final state, the collisional process is enhanced by many orders of magnitude: the laser 'switches on' the collision.

The first observation of such laser-switched collision was reported by Harris and coworkers (Harris *et al. in Tunable Lasers and Applications* (eds Mooradian, Jaeger & Stokseth) 193 (Springer, Berlin, 1976); Falcone *et al. Phys. Rev. A* **15**, 1333; 1977) who studied the laser-induced inelastic collisional excitation of calcium atoms by excited strontium atoms such as



and involved laser intensities of the order of  $1 \text{ MW cm}^{-2}$  for the process to be observable. Their observations confirmed theoretical predictions that for dipole-dipole collisions, the laser-switched collision cross section maximises when  $\omega_L$  is tuned to the interatomic frequency of infinitely-separated atoms. In addition they observed an asymmetric lineshape for the collisional dependence on the laser detuning from exact resonance with  $(\omega_A - \omega_D)$ . Lineshape problems have been analysed by several authors. A recent paper by Harris and White (*IEEE J. Quantum Electronics* **QE-13**, 972; 1977) numerically integrates the coupled equations of motion for the dipole-dipole laser-induced collision first over time, then over impact parameters, and their lineshapes closely resemble those experimentally-observed,



### A hundred years ago

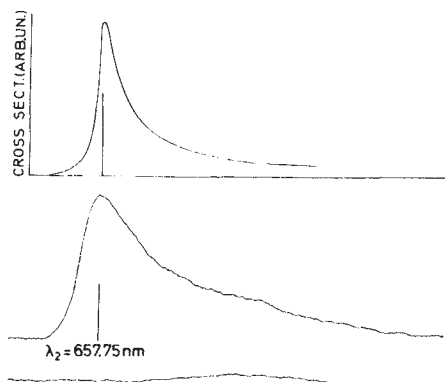
*Brain: a Journal of Neurology* (April, 1878. Part I. To be published quarterly. (London: Macmillan and Co.)

A NEW scientific quarterly has made its debut, entitled *Brain*, a Journal of Neurology, edited by Dr. Bucknill, Crichton-Browne, Ferrier and Hughlings-Jackson, names well known in connection with the physiology and pathology of the nervous system, and supported by an able staff of contributors.

According to the prospectus which has been issued, *Brain* will treat the anatomy, physiology, pathology and therapeutics of the nervous system from the brain, downwards.

From *Nature* **18**, 16 May, 64; 1878.

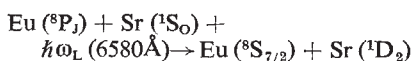
Peter Knight is Jubilee Research Fellow in the Department of Physics at Royal Holloway College.



**Fig. 2** Laser-induced collisional energy transfer spectra; the top curve is theoretical, middle the trace for  $^8P_J$ ,  $J=9/2$ , initially excited europium atoms and the bottom curve the baseline with the laser responsible for Eu excitation blocked. (From Cahuzac & Toschek *op. cit.*)

with a long tail on the 'red' side of the resonance and an abrupt fall on the 'blue' side.

The experiments are complex and laser-switched collisions can be 'mimicked' by quite separate phenomena having similar density and power-dependences which could mislead. Harris and his colleagues initially experienced some of these problems and overcame them in their recent observation of radiative collisions by careful choice of transition and operating wavelength. Confirmation of the existence of laser-switched collisions has now been provided by P. Cahuzac and P. Toschek, with a preliminary announcement at the TICOLS meeting (*Laser Spectroscopy* (eds Hall & Carlsten, (Springer-Verlag, Berlin, 1977)), and now published (*Phys. Rev. Lett.* **40**, 1087; 1978). Cahuzac and Toschek studied the laser-switched collisional excitation of strontium atoms by excited europium atoms irradiated by a nitrogen-pumped pulsed dye laser tunable from approximately 6,400 Å to 6,600 Å at about 800 kW peak power, so that collisions of the type



could be observed. The initially excited europium atoms with angular momentum  $J$  were produced by a separate dye laser and the collisionally-excited strontium atoms detected by observing the decay radiation of the  $5p^2\ ^1D_2 \rightarrow 5p\ ^1P_1$  transition at 6550 Å. The intensity of this decay radiation is a direct monitor of the laser-switched collision and was measured as a function of laser wavelength for initial europium states  $^8P_J$  with  $J = 9/2, 7/2$  and  $5/2$ . Figure 2 shows one of their

laser-switched collision spectra for  $J = 9/2$  and a calculated spectrum for a zero-bandwidth laser based upon numerical integration of the collisional equations of motion. These results, together with those of Harris *et al.* demonstrate the existence of narrow spectra, centred at the  $R=\infty$  inter-atomic wavelengths, with a long tail characteristic of potential energy curve-crossings here generated by photon absorption.

These experiments are important in

that they represent the first observation of the storage and subsequent controlled transfer of energy in a collision. There are obvious applications in chemistry where reactions could be initiated and selectively steered by light absorption, and could be switched on and off in a reproducible fashion. In emission, radiative collisions could enable us to build a laser whose frequency is determined by the energy levels of two different atoms or molecules. □

## Better resolution from Landsat

from J. A. Allan

LANDSAT 3 was successfully launched on 5 March filling the gap left by the ailing Landsat 1 which finally ceased to function in January 1978.

The purpose of the Landsat programme has been to establish whether imagery from orbiting satellites could make a significant



**Fig. 1** Four-frame mosaic of Eastern Massachusetts and Rhode Island taken on 9 March. Notable landmarks include Boston (upper left) Cape Cod Canal (left and above picture centre), Providence RI (lower left), Cape Cod (upper right) and Nantucket Island (lower right). The area was covered by the heavy snow that had fallen about 2 weeks earlier.



contribution to the survey and monitoring of Earth resources. In the six years since the launch of Landsat 1 it has been shown that satellite-borne sensors have an important role in such surveys, but problems and limitations have also been identified. Some would argue that most of the major problems in identifying and predicting phenomena such as land use, cropping and vegetation derive from the 80 metre resolution of the multispectral scanning (MSS) and television camera (RBV) devices on board Landsats 1 and 2.

A number of changes were made in the specifications of the sensors on Landsat 3. In addition to the four MSS bands an additional low resolution infrared/thermal channel has been incorporated. The configuration of the second system, the return beam vidicon (RBV) camera system, has been much more drastically changed. The three camera and three channel RBV multi-spectral capacity of Landsats 1 and 2 has been foregone in favour of higher resolution. Landsat 3 has two identical RBV cameras both with twice the focal length of the cameras on Landsats 1 and 2. The two cameras are mounted side by side and these sense 99 km by 99 km square scenes with a 15% side-lap, giving an image 183 km by 99 km. A mosaic of two such images can be registered to a 185 km by 185 km MSS image. The spectral response of Landsat 3's RBV system is a single broad band from 0.5 to 0.75  $\mu\text{m}$ .

The first Landsat 3 RBV images released by NASA are exciting both to Earth scientists interested in resources studies and to topographic surveyors and photogrammetrists who care about the metric properties of imagery. Whereas on the MSS imagery it has proved difficult to identify consistently roads and sharp boundaries because of the low resolution of the Landsat MSS system, on the new RBV imagery road alignments and junctions as well as land/water divides and even field boundaries can be readily delineated because the spatial resolution of the system is between 40 and 50 m. The intrinsic potential of the new RBV imagery is immense and at the same time its availability will increase the utility of the lower resolution MSS imagery as the RBV imagery will provide information to supplement that recorded on MSS images by overlaying the MSS and RBV records.

Unfortunately the improved resolution is likely to attract loud criticism from many quarters in that the capacity of surveillance is viewed uneasily, especially by the governments of developing countries. It is widely

recognised that there are many military satellites with much higher resolution sensing systems together with more flexibility and manoeuvrability. However, the imagery from such satellites is not available for sale whereas the imagery from the Landsat experiments

is. It will be interesting to see how the new imagery is received by the scientific community as well as by governments. It is likely that the debate will be vigorous and that it will be resolved according to political rather than scientific considerations. □



**Fig. 2** Cape Canaveral, Florida from satellite. Taken 14 March from an altitude of 917 km the photo shows major launching pads at Cape Canaveral (white dots left and slightly above picture centre), the Apollo launching pads (upper left), Shuttle Transportation System runway (far upper left) and several bridges and causeways crossing the Banana and Indian Rivers (upper left corner to lower centre). The bridge in upper left connecting the Kennedy Space Center to the mainland seems to be open to let large ships through. The white dots in the Banana and Indian Rivers are believed to be channel markers, or are dredging deposits. Other notable landmarks include the cities of Cocoa Beach, Merritt Island and Cocoa (centre to left of centre), Port Canaveral (adjacent to Cape Canaveral), and a major highway Route 1 (bottom centre to upper left).

# review article

## Mammals, resources and reproductive strategies

T. H. Clutton-Brock\* & Paul H. Harvey†

*The breeding systems of mammalian species are strongly influenced by the density and dispersion of their populations. These in turn are affected by variations in the distribution of resources. Through quantitative comparisons, it is possible to trace evolutionary relationships between feeding behaviour, population dispersion, breeding systems and morphology.*

FUNCTIONAL explanations of species differences among vertebrates have been bedevilled by the absurd and the fantastic. Thayer's classic suggestion that the pinkness of flamingoes may serve to camouflage them against red sunsets is a well known example, but many hypotheses on a similar level of improbability can be found in the recent literature. The problem is not principally that of armchair theorists getting progressively out of touch with ecological reality. On the contrary, unlikely explanations frequently arise when a scientist explains the occurrence of a trait in a particular species (or of a difference between two species) with which he is closely familiar, without considering the broader distribution of the trait. A good example is provided by the recent finding that sexual dimorphism in size is greater among the larger australopithecines than among the smaller ones<sup>2,3</sup>. Several explanations of this difference have been advanced, but all ignore the fact that there is a widespread tendency among mammals (and among primates in particular) for larger species to show increased size dimorphism<sup>4</sup>. A reasonable explanation of the difference between australopithecines would clearly need to take cognisance of the broader trend.

Functional explanations of species differences are most likely to be reliable when the adaptive significance of a trait is reasonably well understood and accords with its distribution across species. For this reason, we believe that an early step in understanding adaptive variation among mammals should be to investigate distributions of interspecific differences. By this, we do not intend to argue that such studies should supersede attempts to investigate in more detail differences within or between related species. Examination of the precise interrelationships between behaviour and ecology in particular species and broad interspecific comparisons are clearly complementary. Nor do we wish to disguise the problems of investigating the distributions of traits across species: not only may similar traits have different functions<sup>5</sup>, but it is often difficult to interpret relationships between particular traits and ecological variables<sup>6</sup>. As a result, attempts to explain distributions can themselves lead to spurious relationships and absurd explanations.

In this review, we consider four aspects of mammalian biology (population density, group size, breeding system and sexual dimorphism) where it has proved possible to demonstrate systematic associations between species differences and environmental variables. All four are interrelated: the thesis which we shall advance is that variation in the distribution, density and quality of food supplies is largely

responsible for species differences in the density and distribution of mammalian populations; that differences in population density and distribution play an important part in determining differences in breeding systems and that these affect a wide variety of morphological and physiological traits. The recognition of these trends is nothing new: it is clear that sociobiology was well advanced in ancient Greece—"For it is differences in feeding habits that make some animals live in herds and others scattered about; some are carnivorous, some vegetarian, others will eat anything. So, in order to make it easier for them to get these nutrients, nature has given them different ways of life. Again, since animals do not all like the same food but have different tastes according to their nature, so the ways of living of carnivorous animals differ among their different kinds, as do also those of the vegetarian."<sup>7</sup>

In the past, functional approaches to interspecific differences among mammals have been hampered by the lack of relevant theory on which to base predictions, as well as by the paucity of field studies to test them. Over the last half decade the situation has changed. Developments in ecological and evolutionary theory<sup>8-10</sup> make it increasingly possible to predict distributions, while the growing number of species which have been studied in their natural environment allows us to examine the fit between theory and practice. In some cases, relationships are surprisingly close: we have been able to show that over 75% of the variance of average home range size among primate species can be accounted for by two variables, group weight and the proportion of foliage eaten<sup>6</sup>.

Another factor which has hindered functional explanations has been the failure to combine genetic and energetic theory: in our experience it is almost always necessary to consider both when seeking to understand species differences.

### Population density, biomass and home range size

Two energetic principles—truisms, almost—help to explain much about interspecific differences in population distribution among mammals. First, the gross nutritional requirements of an individual increase with body weight but do not do so proportionately. Owing to decreasing heat loss per unit weight in larger mammals, the basal metabolic rate rises as the ~0.75 power of body weight<sup>11,12</sup>. The relative costs of most forms of locomotion also decrease with body size<sup>13,14</sup>. Total energetic requirements may vary widely between phyla, but it is usually safe to assume that absolute energetic requirements increase with body size while relative requirements (that is per unit body weight) decline<sup>15</sup>. Second,

\*King's College Research Centre, Cambridge, UK.

†School of Biological Sciences, University of Sussex, Brighton, UK.



gross interspecific differences in population density and biomass are usually related to differences in the availability of food. It is generally more useful to consider variations in biomass since this takes account of interspecific size differences. Since standing crop of the non-reproductive parts of wild plants (leaf, stem) available to herbivores generally far exceeds that of reproductive parts (flowers and fruit)<sup>16</sup>, we can expect to find the highest biomasses among relatively unselective grazers and the lowest among specialised frugivores. Similarly, because the biomass and energy available from plant matter almost always greatly exceeds that of animal prey, we can expect insectivores and carnivores to show lower biomasses than folivores and frugivores.

As might be expected, total herbivore biomass is closely related to variation in primary production: it is greater in the tropics or subtropics than in temperate or arctic regions<sup>17</sup>. In the tropics and subtropics both biomass and primary production are closely related to annual precipitation and evapotranspiration<sup>18</sup>, although other factors including soils<sup>19,20</sup>, altitude<sup>21,22</sup> and human interference<sup>23</sup> may also be important.

In addition, species differences in population density and biomass are related to food availability. Since large-bodied species require absolutely more energy than small ones, their population densities should usually be lower: this can be shown to be the case among ungulates and primates<sup>6</sup>, although the relationship is complicated by an association between small body size and highly selective feeding<sup>24</sup>. Species feeding on relatively abundant foods show higher population density and biomass than those feeding on less common ones: biomasses of folivorous primates are higher than those of frugivores<sup>6</sup>, while among African ungulates, grazers show higher biomasses than browsers. The biomasses of carnivores are generally far lower than those of herbivores or frugivores<sup>25-27</sup>.

Translated into behavioural terms, this means that large-bodied species (or those living in large groups) have absolutely larger home ranges or territories than smaller species (or those living in small groups) and that home range size is related to diet type. Relationships between home range size, diet and body weight have been demonstrated in small mammals<sup>28</sup>, primates<sup>29,30</sup>, carnivores<sup>31</sup> and ungulates (T.H.C.-B. and P.H.H., in preparation). Home range size does not always increase in proportion to body weight. Among herbivorous and omnivorous small mammals, larger species have relatively small (per unit body weight) home ranges, and home range size increases with body weight to about the 0.65 power. In contrast, among carnivores, relative home range size increases with body weight (power of 1.41; ref. 31), and a similar trend is found among predatory birds<sup>31</sup>. The difference between carnivores and other dietetic groups may arise because increasing body size in carnivores restricts the size range of prey that are economical to hunt, leading to a decrease in the biomass of available prey as body size increases<sup>30,31</sup>. The relative decrease in home range size with increased body weight among small mammals has been explained as a result of the decline in relative metabolic costs with increased body weight<sup>28</sup>.

The dispersion of resources also affects home range size. Where food supplies are strongly clumped, widely dispersed or unpredictable in abundance, mammals occupy large home ranges<sup>32,33</sup>. In contrast, where supplies are evenly dispersed and predictable, small home range size results and the defence of feeding territories becomes practicable if food density is high enough<sup>34</sup>.

### Group size and patterns of population dispersion

The abundance and distribution of food supplies affect population dispersion. While grouping can serve many dif-

ferent functions in mammals<sup>30</sup>, species differences in group size seem to be best predicted by variation in the disadvantages (rather than the advantages) of grouping<sup>30</sup>. Group feeding is likely to lead to feeding interference and a decline in the rate of food intake<sup>24,35,36</sup>. Feeding interference will be most pronounced where individuals eat out (or defend) whole food sources, forcing latecomers to search elsewhere, and where the distance between food items is large<sup>24</sup>. In contrast, it will be least important where individuals only take a proportion of the food available at each source (as in many grazing species) and where the distance between food items is small. Among the African ungulates, highly selective grazers and browsers (duiker, klipspringer, oribi, gerenuk, kudu) generally live alone or in small groups, whereas less selective grazers (wildebeest, zebra, topi, buffalo) often feed in large herds<sup>24,37</sup>. Similar trends occur among pachyderms<sup>38</sup>, cervids (T.H.C.-B. and P.H.H., in preparation) and herbivorous marsupials<sup>39,40</sup>.

The dispersion of food sources can also influence population dispersion. Species which depend on food sources occurring in large, evanescent clumps often feed in big groups and rarely defend territories<sup>8,24,32</sup>. In contrast, where the distribution of food supplies is regular enough and food density high enough to permit territory defence<sup>34</sup>, group size may be constrained by the inevitable decline in the efficiency with which other animals can be excluded as group weight (and hence territory size) increases<sup>33,41</sup>.

Many other environmental factors apparently affect group size. Among primates<sup>42</sup>, nocturnal species usually feed alone, irrespective of their diet type: one possible explanation is that most small nocturnal primates rely on crypsis for defence<sup>30,43</sup> and that this precludes social feeding. And among carnivores, social feeding is often an adaptation to large prey size and tends to occur among species which feed primarily on prey whose body size is larger than their own<sup>26,44</sup>.

### Breeding systems and constraints on polygyny

Because the abundance and distribution of food resources affect population density and dispersion, they also have profound effects on the breeding systems which different animals adopt<sup>45</sup>. Two recent developments in evolutionary theory are of particular importance in understanding the functional significance of breeding systems. The first is the extension of the concept of 'Darwinian fitness' (the number of direct descendants that an individual leaves in subsequent generations) to that of 'inclusive fitness' (the amount of an individual's genotype which is passed on to subsequent generations), a development due largely to W. D. Hamilton<sup>46</sup> and often referred to as 'kin selection'. The second is the reinterpretation of the Darwinian theory of intra-sexual selection<sup>47,48</sup>. The first has been successfully used to explain a wide variety of examples of cooperative breeding by related animals, from communal suckling in lions<sup>49</sup> to the evolution of sterile casts in the social hymenoptera<sup>46</sup>. The second is of particular importance in understanding mammalian breeding systems. Trivers<sup>48</sup> envisages individuals of each sex as having limited resource budgets which they can invest in their offspring (or other relatives). One sex generally invests more than the other: in mammals this is usually the female since she suffers the costs of gestation and lactation (the reasons for this initial asymmetry in investment have been discussed elsewhere<sup>48,50</sup>, but are not directly relevant to the present argument). As a result, female mammals cannot produce and rear offspring at the rate at which males can father them. Consequently, while the reproductive success of females will usually be limited by the number of young they can produce and raise, that of males will often be limited by factors affecting the number of females they can fertilise.

The theory explains why, among mammals, polygyny is common and polyandry rare or nonexistent: the advantages

to a female of maintaining mating access to several males will, in almost all circumstances, be less than the advantages to a male of maintaining mating access to several females<sup>48,50</sup>. Through its effect on the distribution of females, the distribution and density of resources plays an important part in limiting the degree of polygyny which can evolve. Early interpretations of mating systems<sup>51-53</sup> suggested that the occurrence and extent of polygyny is limited by female choice, and that unmated females will only breed polygynously when they improve their fitness by doing so (for example, where a successful male defends access to resources adequate for several females and their young). In a recent review, Emlen and Oring<sup>54</sup> extend this hypothesis, suggesting that polygyny will occur either where males can monopolise resources sufficient to attract several females ("resource defence polygyny") or where they can defend groups of females ("female defence polygyny"). The feasibility of defending either resources or females will be affected by their distribution in time and space as well as by the amount of time and energy which the male can afford to devote to defence of mating access.

The argument successfully merges evolutionary and ecological theory. An individual male may be able to defend access to resources adequate for several females either where resources are evenly dispersed and their density is high, or where resources show some degree of clumping but are concentrated and predictable enough to permit several females to depend on them throughout the year. Examples of this first situation are the harem groups monopolised by territorial males in many South-East Asian langurs<sup>30</sup>. An example of the second is the yellow-bellied marmot, where males defend areas of rock outcroppings used as wintering sites by several females and are thus able partially to control breeding access<sup>55-59</sup>.

Where resources are evenly distributed but sparse, females will be widely dispersed and males may be unable to monopolise access to more than one female: probably for this reason, extreme polygyny is rare among the smaller carnivores and insectivores<sup>50</sup>. An association between low density food supplies and monogamy has been suggested both in birds<sup>53</sup> and primates<sup>30</sup>. In the latter group, Clutton-Brock and Harvey<sup>30</sup> suggest that monogamy occurs where males cannot defend resources adequate for several females either because female size (and hence nutritional requirements) is large or where the biomass of available food supplies is low. In some monogamous species (as in the gibbons and siamangs) the mated pair and their dependent offspring travel and feed together while in others (including many monogamous ungulates), the male and female spend most of their time apart. The latter seems to apply either in species where feeding interference is intense because of the nature of food sources<sup>62</sup> or where individuals rely on crypsis to avoid predators.

Finally, where resources are heavily clumped and clumps are unpredictable or widely dispersed, males will be unable to defend resources adequate for several females. In this situation they may either defend groups of females (as in patas monkeys or hamadryas baboons) or, where the size of female groups is large and they are difficult to defend, accept the presence of other males and attempt to maintain priority of access to receptive females (as among most baboons and macaques). In these cases, the numbers of females which a male can monopolise will be affected by the density of resources as well as by the extent of reproductive synchrony among females: where conceptions are highly synchronised and the duration of courtship is long, males will be less likely to monopolise access to several females successfully<sup>54</sup>. For example, almost all strongly polygynous pinniped species copulate and give birth on land, where females are closely spaced and easily defended<sup>63-65</sup>. In contrast, among species which copulate primarily in the water (such as the common seal, *Phoca vitulina*, and the

monk seals) extreme polygyny has not evolved, probably because aquatic territories are difficult to defend<sup>65</sup>. While most pinnipeds have well defined breeding seasons, synchrony is most marked among species which breed on pack ice where most matings occur within a 10-day period. Extreme polygyny is also absent in this group, perhaps both because females are dispersed and because breeding is highly synchronised<sup>64,65</sup>.

Even where the distribution of females or resources provides the potential for polygyny, males will not always be able to take advantage of it<sup>54</sup>. This will be the case where successful rearing of offspring requires paternal as well as maternal care, when the number of females they breed with may be limited by the number of offspring they can look after<sup>48,50</sup>. While paternal care is closely associated with monogamy among mammals<sup>66,65,30</sup>, it may take a number of different forms. In the majority of monogamous species, males help to defend food reserves used by their mates and offspring<sup>66</sup>. In some arboreal or volant mammals, males also help carry the young. For example, among primates, male marmosets, tamarins, night monkeys, titis and siamangs carry the infant(s) for much of the day<sup>67</sup>. All these species usually breed monogamously and similar behaviour is not recorded among polygynous primates, though males may carry infants and frequently protect them<sup>68</sup>. Not all monogamous primate fathers regularly carry their young, but they tend to do so where the weight of the young is large relative to female weight<sup>66</sup>. Finally, in some mammals (as in many birds) the male helps the female to bring food to the young and/or mother. This tends to occur where food is energetically costly to collect and where litter size is large: for example, it is common among monogamous canids<sup>69,66</sup>. Monogamy developing for this reason alone is most likely to be found in relatively stable environments where intraspecific competition is intense<sup>9,10</sup>.

The difficulty with explaining monogamy as the consequence of the need for paternal care is that paternal care is likely to evolve in circumstances in which polygyny is prevented by other factors. In an evolutionary timespan, it is as possible that the presence of extensive paternal care (and even of increased neonatal weights and larger litter sizes) in the monogamous primates we have listed is a product of the fact that males breed monogamously for other reasons as that the need for paternal care requires monogamous breeding. There is no obvious way out of this problem.

Functional interpretations of breeding systems still present many difficulties. Partly because many different ecological variables are involved and partly because several of these cannot be measured accurately (in particular, the extent to which food supplies are clumped) it is seldom possible to make useful quantitative comparisons. As a result, arguments inevitably rely on selected examples rather than systematic analyses. Finally, there are many unanswered questions: while, in outline we can account for the distribution of monogamy versus polygyny, it is often difficult to explain species differences in the degree of polygyny<sup>30</sup>. Nor do we understand why, in some species, breeding males tolerate the presence of peers whereas in others they do not<sup>30</sup>.

## Breeding competition and sexual dimorphism

The breeding system adopted is known to affect the degree of intrasexual competition for mating access<sup>70</sup>: in strongly polygynous species, we should expect competition between males for access to females to be intense, while in monogamous species it should be reduced or absent<sup>10,70</sup>. Consequently, we might expect traits affecting competitive ability to be better developed in males of species which usually breed polygynously than in those that usually breed monogamously<sup>48</sup>.



Systematic investigation of sexual dimorphism among primates confirms this prediction. In polygynous species, males are larger, relative to female size, than in monogamous ones<sup>1</sup>, show greater development of pelage used in defence or display<sup>71</sup> and have relatively larger canine teeth for their body weight<sup>72</sup>. A similar trend occurs among ungulates where males of polygynous species typically have bigger horns or antlers than males of monogamous species<sup>24</sup>.

While the gross distribution of sexual dimorphism conforms with the pattern predicted by sexual selection theory, it is clear that the trait has also been moulded by many other evolutionary and ecological pressures. In a number of bird species, selection has apparently favoured sex differences in body size or feeding apparatus which allows the sexes to diverge in food choice and thus to utilise food supplies more efficiently<sup>73,74</sup>. Such a process may also help to explain the presence of marked sexual dimorphism in some mammals which are not strongly polygynous<sup>75</sup>. Similarly, since increased body size permits a larger feeding radius from some fixed point such as a burrow or nest, selection may favour increased body size in the sex which is responsible for feeding the young<sup>76</sup>. Both mechanisms would be most likely to be found in species whose searching or pursuit costs are high<sup>77</sup> and should, for this reason, be most likely to occur among predators.

Ecological factors may also constrain sexual dimorphism. The most extreme cases of sexual dimorphism among mammals far exceed birds<sup>78</sup>, presumably because the restrictions on increased weight are reduced in ground dwelling species. Similarly, among primates, dimorphism in body weight is greater in terrestrial than arboreal species, probably because increased weight does not restrict the male's access to food resources growing on fine branches or terminal twigs<sup>4</sup>.

Selection pressures acting on females can also have profound effects on sexual dimorphism. Since body size is positively related to maturation age in many species, selection for early maturation in females (perhaps associated with fluctuating environments) may lead to a decrease in female body size, while large male body size may be maintained by intra-sexual selection<sup>58,79,80</sup> thus producing marked sexual dimorphism in species that are only slightly polygynous. Conversely, selection for increased litter size or neonatal weight may lead to increased female size<sup>81</sup>: large mothers can produce bigger litters (or offspring). The breeding system can affect the breeding access of females to males and this, too, may effect selection pressures operating on sexual dimorphism. In some primate species, females show pronounced swelling of the perineal or para-callosal areas which signal their sexual state<sup>82</sup>. The distribution of swellings is not obviously associated with either environmental or taxonomic differences and has puzzled primatologists since Darwin: "In my *Descent of Man*" he wrote "no case interested me and perplexed me so much as the brightly coloured hinder ends and adjoining parts of certain monkeys". Recent studies show that, with one exception (the hamadryas baboon), the trait occurs in species where females have mating access to several males (although only a proportion of such species have sexual swellings) and suggests that swellings may have developed to attract the attention of males and increase a female's chance of being mated by a high-ranking male<sup>67</sup>. On a similar line of argument, Cox and LeBoeuf<sup>84</sup> suggest that the vociferous complaints of female elephant seals which are mated by subordinate males may serve to increase the chance that they will be mated by a dominant animal.

In yet other cases, competition with males may lead to selection for improved competitive ability among females and thus to reduced dimorphism<sup>85</sup>. Among the Cervidae, female reindeer and caribou are unique in possessing a large pair of antlers and in forming big bisexual herds including large numbers of males<sup>86</sup>. It seems likely that large herd

size may intensify feeding competition between males and females, and that females have developed antlers to allow them to compete successfully with males. In a number of primates which live in large, mixed-sex groups, females may suffer regular feeding interference from males (Packer and Pusey, in preparation). Perhaps because of this, females possess relatively large canines (for their body size) in primate species which form multi-male groups than in those which live in harem groups where the adult sex ratio is strongly biased against males (P.H.H., M. Kavanagh and T.H.C.-B., in preparation).

We have concentrated, in this section, on sexual dimorphism in body size partly because enough data are available for quantitative comparisons and partly because it provides a good example of the interaction of genetic and energetic influences. It is clear that interspecific variation in breeding systems will also prove to be associated with a wide variety of other morphological and physiological differences, including variation in social relationships<sup>87</sup> and maturational processes<sup>79,80</sup>.

We must end with two caveats. When explaining interspecific differences, it is almost always impossible to test functional arguments by experiment and we rely on correlations and associations. As we have argued, causes are often difficult to distinguish from effects. In some cases it is not of critical importance to distinguish the direction of causality. For example, large sacculated stomachs equipped with microbial symbionts permit herbivores to use forage with a high cellulose fraction<sup>15</sup>. It is not usually important to decide whether, in an evolutionary perspective, forage quality controls digestive anatomy or vice versa. In others, our understanding of causality will have a profound effect on the explanations of distributions. In some of these, the reasons for an association are fairly obvious: it is, for example, more likely that differences in sexual dimorphism are caused (in part) by differences in breeding systems than vice versa. In yet other cases, such as the relationship between monogamy and paternal investment, no such assumption can be made. It is impossible to generalise about relationships of this kind and each case needs to be considered separately. The usual assumption made by evolutionary ecologists (albeit tacitly) is that the feeding niche occupied by a species (and the morphological, physiological and behavioural adaptations which affect food exploitation directly) is most likely to constrain evolutionary changes in other variables<sup>8</sup>.

Second, while we have concentrated throughout this review on generalisations, there are many exceptions to every generalisation that we have cited. We do not believe that their existence reduces the value of attempting to generalise. On the contrary, only when we understand the ground rules of mammalian adaptation shall we be able to ask sensible questions about the exceptions.

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## articles

# Equivalent circuit analysis of neutral wind effects on equatorial electrojet

C. A. Reddy & C. V. Devasia

Space Physics Division, Vikram Sarabhai Space Centre, Trivandrum 695022, India

*The generation of electric fields and currents by local winds in the equatorial electrojet is treated by an equivalent electric circuit method. Apart from its mathematical simplicity, the method provides a full and clear view of the physical processes involved in the generation of currents and electric fields by shearing winds, and thus permits a straightforward interpretation of the main effects of winds. The method is valuable for the theoretical analysis of the wind-related electrostatics of the equatorial electrojet.*

THE effects of local winds on the ionospheric dynamo-region currents at or near the magnetic dip equator have previously been considered negligible because the effect of the wind is normally cancelled out by the polarisation electric field set up by the wind. Recently, the local wind effects on the equatorial electrojet have

received increasing attention<sup>1–6</sup>, as it was realised that height-varying winds, in contrast to the steady winds considered earlier, could have significant electrodynamic effects in the equatorial electrojet region. Kato<sup>1</sup> pointed out that the polarisation electric field set up by wind motion at the magnetic equator would be partially short-circuited by the currents flowing across the curved magnetic field lines in the dynamo region away from the equator. He showed this effect using an equivalent circuit representation of the dynamo region at the magnetic equator and its coupling, through the highly conducting magnetic field lines, to the conducting region away from the equator. His treatment of the problem was incomplete, however, to the extent that the contribution of the wind-induced dynamo fields in the same dynamo region where the short-circuiting currents flow was not taken into account. On the other hand, Forbes and Lindzen<sup>3</sup> considered a three-dimensional model of the equatorial electrojet in which the effects of shearing neutral winds were included. They concluded that even a two-dimensional model of the electrojet would



essentially reproduce the effects of the shearing winds on the height-structure of the electrojet current density. We obtained similar results<sup>6</sup> independently through a theoretical study in which an equivalent circuit method was used to calculate the effect of shearing winds on the height structure of the equatorial electrojet at different latitudes. This theoretical study was carried out to clarify the possible role of shearing winds in producing the short period fluctuations of the equatorial electrojet as observed with a VHF backscatter radar at Thumba<sup>7</sup>. The fluctuation amplitudes are typically 10–20% of the total electrojet current intensity and the quasi-periods are in the range 20–40 min, indicating a possible gravity wave origin.

A recent critical analysis has revealed that application of the electric circuit theory provides a clear insight into the interesting physical processes involved in the generation of currents and electric fields by shearing winds. In addition, the method enables a straightforward interpretation of many numerical computational results of earlier workers. We present here the theoretical treatment of the problem in terms of the equivalent circuit method, and its usefulness in dealing with different aspects of the equatorial electrojet is demonstrated.

## Physical basis of the equivalent circuit method

To understand the physical processes involved in the generation of electrical currents in the equatorial electrojet by shearing neutral winds we consider Fig. 1. The effect of a steady wind ( $\mathbf{U}$ ), with no variation with altitude or latitude, is shown in Fig. 1a, where  $\mathbf{U}$  is shown as eastwards, for convenience. The ions are dragged along by the neutrals; the ion velocity is equal to the wind velocity at the lower altitudes ( $< 120$  km) but it becomes a progressively decreasing fraction of the wind velocity at the higher altitudes due to the decreasing collisional friction. If  $\mathbf{V}_i$  is the wind-generated ion velocity, then  $\mathbf{e} = (\mathbf{V}_i \times \mathbf{B})$  is the upwards Lorentz force acting on the ions. As the Lorentz force causes upwards motion of ions (and hence an upwards current), the dynamo electric field  $\mathbf{e}$  is equivalent, in its effect on ion motion, to that of a battery whose conventional positive terminal is on the upper side. The Pedersen drift of ions due to  $\mathbf{e}$  can result in a charge accumulation and a downward polarisation electric field  $\mathbf{E}_p$ . In the absence of a leakage path for the ions drifting upward, the equilibrium condition of  $\mathbf{E}_p = -\mathbf{e} = -(\mathbf{V}_i \times \mathbf{B})$  is quickly established. In this equilibrium condition, the vertical motion of the ions is inhibited, but their horizontal motion with velocity  $\mathbf{V}_i$  is maintained.

Because the electrons have a negligibly small Pedersen mobility, they are not affected by  $\mathbf{U}$ . However, they are subjected to a Hall drift in the eastwards direction due to  $\mathbf{E}_p$  and their drift velocity  $\mathbf{V}_e$  is given by

$$\mathbf{V}_e = \frac{\mathbf{E}_p \times \mathbf{B}}{B^2} = -\frac{(\mathbf{V}_i \times \mathbf{B}) \times \mathbf{B}}{B^2} = \mathbf{V}_i \quad (1)$$

where  $\mathbf{B}$  is the geomagnetic field. Thus the electrons move with the same velocity as the ions and there is no current flow due to the wind motion, as long as the wind has no variation with altitude or latitude. This is the physical basis for neglecting the effect of local winds on the equatorial electrojet.

It is important, however, that the plasma as a whole moves with the steady wind velocity at and near the magnetic equator and this aspect has many implications, though it is not discussed further here. Furthermore, the generation of differential motion of ions and electrons requires that the polarisation field  $\mathbf{E}_p$  be discharged through a current flow across the magnetic field lines. It is shown below that height-varying winds can lead to such short-circuiting currents.

Figure 1b shows an electric circuit representation which is useful for gaining an insight into the physical processes of wind effects, and also for a quantitative theoretical treatment under

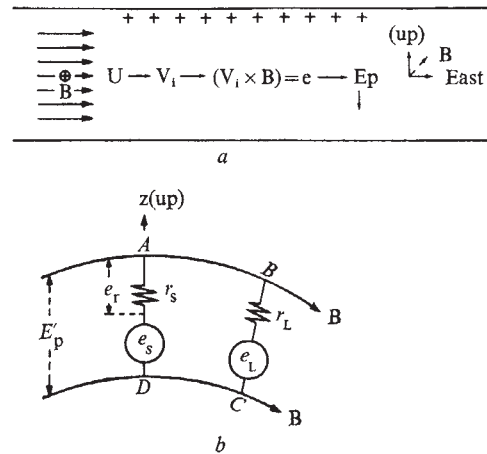


Fig. 1 a, Generation process of the electrodynamic and polarisation electric fields at the magnetic dip equator by a steady wind. b, Equivalent circuit method for treating the effect of a shearing wind near the magnetic equator.  $\mathbf{U}$  is perpendicular to  $ABCD$ .

different wind conditions.  $ABCD$  represents a section of the vertical magnetic meridional plane;  $\mathbf{e}_s$  is the dynamo electric field due to a local wind and  $r_s$  is the internal resistance in an element of unit volume in the 'source' region (say, at the equator), while  $\mathbf{e}_L$  and  $r_L$  are the corresponding parameters in a distant 'load' region (say, a few degrees away from the equator); the two volume elements are connected by the slightly curved and highly conducting magnetic field lines,  $AB$  and  $DC$ . The two volume elements are located at different altitudes, and  $r_s$  or  $r_L$  represents the resistance of the respective volume element for Pedersen (ion) currents across the magnetic field lines. In the subsequent use of the equivalent circuit method, the dynamo electric field  $(\mathbf{V}_i \times \mathbf{B})$  is represented by the equivalent battery e.m.f.  $\mathbf{e}_s = -(\mathbf{V}_i \times \mathbf{B})$  in the unit volume element. This is done for convenience of treatment in the equivalent circuit method, but the e.m.f. and electric field are different in dimensions though they are equal in value for a volume element of unit side length. Any other potential difference or electric field across the magnetic field lines is taken to be positive if it has an upward-directed component, while the opposite is true of the  $(\mathbf{V}_i \times \mathbf{B})$  field. With the above convention,  $\mathbf{E}_p = \mathbf{e}_s$ , rather than  $-\mathbf{e}_s$ , with reference to Fig. 1a. Moreover,  $\mathbf{e}_s$  or  $\mathbf{E}_p$  is essentially vertical in the whole of the equatorial electrojet and it is exactly so at the magnetic equator as long as the effects of east-west winds only are considered (as here). Hence the vector notation for  $\mathbf{e}_s$  and  $\mathbf{E}_p$  is dropped in the rest of the paper. We can now discuss the currents in the source region in different wind conditions, in terms of the equivalent circuit representation (Fig. 1b).

(1) Steady wind: here,  $\mathbf{e}_s = \mathbf{e}_L$ ,  $i_s = 0$  and  $\mathbf{E}_p = \mathbf{e}_s$ . Consequently, the wind-generated east-west current ( $j_w$ ) is zero as discussed earlier with reference to Fig. 1a.

(2) Wind decreasing with decreasing altitude but no change of sign: here,  $|e_L| < |e_s|$  and a current  $i_s = (e_s - e_L) / (r_s + r_L)$  flows through the source region and causes a potential drop  $e_r = i_s r_s$ . The resultant potential difference between the terminals  $A$  and  $D$  is  $E_p + e_r$ . In the case of a steady wind decreasing with decreasing altitude but no change of sign,  $e_L$  and  $e_s$  will have the same sign ( $-ve$  for eastward wind and  $+ve$  for westward wind) and  $\mathbf{E}_p$  and  $\mathbf{e}_r$  will have opposite signs; therefore, the resultant potential difference ( $E_p'$ ) between the terminals  $A$  and  $D$  is given by

$$E_p' = \pm(E_p - e_r) = \pm(e_s - e_r) \quad (2)$$

where the  $-ve$  sign applies in case of an eastwards wind.

Thus the wind-generated polarisation field ( $\mathbf{E}_p$ ) is partly short-circuited and reduced in value by  $e_r$ . Correspondingly, the electron velocity is reduced to a value lower than that of the ion velocity and this differential motion of ions and electrons gives

rise to the wind-generated current. The differential velocity of electrons relative to the ions is directly proportional to  $e_r$ ; hence the larger the vertical current, the larger is the potential drop in the source region, and the larger is the wind-generated current in the east-west direction.

Though we have considered the source region to be located at the equator, the 'source' and 'load' regions can be interchanged without loss of generality.

(3) Wind increasing with decreasing altitude but no change of sign: here  $|e_L| > |e_s|$  and the current  $i_s$  will be in a direction opposite to that of case (2). However, the resultant potential difference across the terminals  $A$  and  $D$  is now given by

$$E_p' = \pm(e_s + e_r) \quad (3)$$

the negative sign applies in case of an eastwards wind, as before. In this case, the electrons will move faster than ions in the direction of the wind, the differential velocity being proportional to  $e_r$ .

(4) Wind with large shear and reversal of direction: if we consider the case of east-west winds of equal magnitude but with opposite directions in the source and load regions, then  $e_L = -e_s$  and

$$i_s = \pm 2e_s/(r_s + r_L) \quad (4)$$

where the +ve sign applies when the wind is eastwards in the source region. The resultant polarisation field  $E_p'$  in this case is given by

$$E_p' = -(e_s - e_r) \quad (5)$$

$$\text{where } e_r = \pm 2e_s r_s/(r_s + r_L) \quad (6)$$

$$\text{If } r_L = r_s, \text{ then } e_r = \pm e_s \text{ and } E_p' = 0 \quad (7)$$

In this condition, the electrons remain immobile due to the complete short-circuiting of the wind-generated polarisation field, but the ions move with the wind. Therefore, the wind-generated current in this case consists of ions moving with the wind, while the electrons remain stationary (in the absence of other electric fields). In the frame of reference of the moving ions, the current can also be visualised as an electron current, the electron velocity being the Hall drift velocity due to  $e_r$ .

(5) The extreme case of a very large wind with reversed direction in the 'load' region: it is useful to consider an extreme case in which  $e_L = -3e_s$  and  $r_L = r_s$ . In this case,

$$i_s = \pm 2e_s/r_s; e_r = \pm 2e_s \quad (8)$$

The +ve sign applies when the wind is eastwards in the source region. The resultant polarisation field  $E_p'$  is then given by

$$E_p' = -(e_s - e_r) = \pm e_s \quad (9)$$

where the +ve sign applies for an eastwards wind in the source region. Thus, in this case an eastwards wind generates an upward electric field and drives the electrons in the westwards direction with velocity  $V_e = -V_i$ , while the ions move eastwards with velocity  $V_i$ . In effect, the drift velocity of electrons relative to ions  $2V_i$  and this is precisely the Hall drift velocity of electrons due to the electrostatic field  $2e_r$  which is vertically upward for the case of an eastwards wind in the source region followed by a westwards wind in the 'load' region. In this case, the wind-generated current is double of that in case (4).

## Contrast with electric field-generated currents

It is interesting to note the contrast in the mechanisms of east-west current generation by winds and electric fields in the equatorial electrojet. The east-west electrostatic field  $E_y$  in the electrojet generates a vertical Hall drift of electrons relative to ions. When this vertical electron current is inhibited, charge accumulation results in a large vertical polarisation electric field which in turn causes a Hall drift of electrons in the east-west direction with large speeds (several hundred  $\text{ms}^{-1}$ ) relative to ions which remain almost stationary in the absence of winds. In contrast to the above process, the current generation by shearing winds takes place essentially through the inhibition of the vertical polarisation field by the maintenance of a large vertical current. The larger the wind shear, the larger is the vertical current and the larger is the east-west current due to the winds.

Also the current in the circuit is carried by the drifting ions in the path across the magnetic field lines (along  $AD$  and  $BC$  in Fig. 1b) and by the more mobile electrons along the field lines (along  $AB$  and  $DC$ ). Thus the analogy with an electric circuit, in which batteries of finite internal resistance are connected together by wires, is complete.

## Application

The derivation of the general equations for the wind-generated polarisation field and current in the equatorial electrojet can be done through an extension of the equivalent circuit method described above. The equivalent circuit for the general case of any arbitrary distribution of east-west wind vectors and Pedersen conductivities in the magnetic meridional plane is shown in Fig. 2.  $AB$  and  $DC$  are the highly conducting magnetic field lines bounding, in the vertical plane, the multitude of adjacent unit volume elements with different values of east-west wind and Pedersen conductivity. The wind-generated e.m.f.s are denoted by  $e_1, e_2, e_3, \dots, e_n$ , while the resistances of the unit volume elements are denoted by  $r_1, r_2, \dots, r_n$ .  $e_s$  and  $r_s$  denote the 'source' region e.m.f. and resistance respectively. The source region can be any unit volume element located at any altitude and latitude in the electrojet, but for convenience we choose the source region to be located at the magnetic equator.

In Fig. 2,  $R_L$  is the effective load resistance connected across the source region and it is the resultant of the parallel combination of  $r_1, r_2, \dots, r_n$ .

$$1/R_L = \sum_{i=1}^n (1/r_i) \quad (10)$$

The current  $(i_s)_s$  through  $r_s$  due to  $e_s$  alone is given by

$$(i_s)_s = e_s/(r_s + R_L) \quad (11)$$

Similarly, the current through  $r_s$  due to any other e.m.f.  $e_m$  is given by

$$(i_s)_m = -\frac{e_m R_L}{r_m + R_L} / r_s \quad (12)$$

Adding up all currents (due to all e.m.f.s) flowing through  $r_s$ , we obtain

$$i_s = (i_s)_s + \sum_{i=1}^n (i_s)_m \quad (13)$$



$$= \frac{e_s}{r_s + R_L} - \frac{R_L}{r_s} \sum_1^n \left[ e_m / (r_m + R_L) \right] \quad (14)$$

$$e_r = i_s r_s = \frac{e_s r_s}{r_s + R_L} - R_L \sum_1^n \left[ e_m / (r_m + R_L) \right] \quad (15)$$

$i_s$  is the net current through  $r_s$ , and  $e_r$  is the potential drop. The signs of  $i_s$  and  $e_r$  depend on the signs of  $e_s$  and  $\sum e_m / (r_m + R_L)$ . As before, an eastwards wind in any region leads to a negative sign the equivalent battery e.m.f.  $e_m$  or  $e_s$ . The polarisation field in this general case, is

$$E_p' = \pm (e_s - e_r) \quad (16)$$

with the same sign convention as for equation (3).

In most situations,  $R_L \ll r_m$  or  $r_s$ , and equation (15) can be simplified to

$$e_r \doteq e_s - R_L \sum_1^n (e_m / r_m) \quad (17)$$

When the wind has no variation with altitude or latitude,  $e_m = e_s$  everywhere and equation (17) above reduces to

$$e_r \doteq e_s - e_s R_L \sum_1^n 1/r_m = 0 \quad (18)$$

in view of equation (10) for  $R_L$ . Correspondingly, the resultant perpendicular electric field  $E_p'$  is:

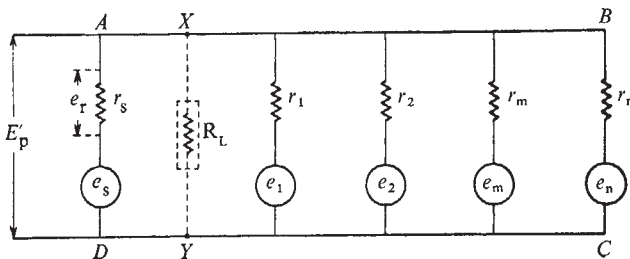
$$E_p' = \pm e_s \quad (19)$$

Thus, from equations (18) and (19) we find that a constant wind does not produce a current in the equatorial electrojet.

The wind generated east-west current ( $j_w$ ) is given by

$$j_w = \sigma_H e_r \doteq \sigma_H \left[ e_s - R_L \sum_1^n (e_m / r_m) \right] \quad (20)$$

**Fig. 2** Electric circuit representation of the electric fields and currents in the magnetic meridional plane due to shearing neutral winds in the equatorial electrojet.



## Discussion and conclusions

Even without numerical computations, equations (17) and (20) enable us to interpret the physical conditions under which the shearing winds are most effective in generating east-west currents at the magnetic equator: first, the wind should have a change of direction within a short height interval, and preferably, the reversed wind at the lower altitude (away from the equator along the same field line) should have a much larger amplitude than that at the equator; second, the large amplitude wind at the lower altitude should be located in a region of low  $r_m$  (high Pedersen conductivity). However, low values of  $r_m$  also lead to a low  $R_L$  across any flux tube and this tends to reduce the contribution of the second term. One can also argue that the source region dynamo field  $e_s$ , the resistance  $r_s$  and also  $r_m$  should be large across the greater part of the flux tube, with a low value of  $r_m$  in a limited region where a large wind of reversed direction gives rise to a large value of  $e_m$  with a sign opposite to that of  $e_s$ . But as  $e_s$  becomes large, it requires a larger value of  $e_m$  of opposite sign to drive a current through  $r_s$  in the right direction.

The above discussion brings into focus three pertinent points: (1) only winds with large shears can produce significant current at the magnetic equator; but the currents, being directed oppositely at different altitudes in such case, lead to comparatively small values of height-integrated current intensity; (2) in spite of the frequent presence of shearing winds, significant height-integrated currents can be generated only on a limited number of occasions when the wind shears of right magnitude, sign and height structure exist at the appropriate altitudes; (3) assumed wind models which can produce appreciable currents have to be critically assessed for their realism.

Many computational results of earlier workers can be explained by equations (17) and (20). First, equation (18) of Richmond<sup>2</sup> is the same as equation (20), because  $e = V_\phi \times B$ ,  $r_m = 1/\sigma_1$  and  $R_L = (\int \sigma_1 dS)^{-1}$ ; as such it is an approximate form of an exact equation which should use equation (14) for  $i_s$  in calculating  $e_r$ . However, the approximation involved in equation (20) and Richmond's equation (18) is valid. Second, the calculations of Kato<sup>1</sup> and Ananda Rao<sup>4,5</sup> show that the efficiency of the wind in reducing the polarisation field  $E_p$  increases with increasing shear. The physical basis of this process has been explained with reference to Fig. 1b, and the result, summarised by equation (17), has been discussed already. Many detailed features of their numerical results can be identified as the natural consequences of the physical interpretation of equations (17) and (20). Third, the basis for the result of Forbes and Lindzen<sup>3</sup> that a larger vertical current leads to a larger east-west current in the equatorial electrojet is readily understood in the present method: the vertical current, consisting of the Pedersen drift of ions across the magnetic field lines, partially discharges the polarisation electric field set up by the wind; and the east-west current is proportional to the 'discharged' part of the polarisation field. Therefore, a wind structure producing a larger vertical current results in a larger east-west current.

From the present method, one can also understand the physical basis of the result<sup>3</sup> that a two-dimensional model essentially reproduces the results of the three-dimensional model as far as the effects of neutral winds are concerned. As the vertical current and the associated potential drop ( $e_r$ ) determine the resultant polarisation field ( $E_p'$ ) and the consequent east-west current density ( $j_w$ ), a correct description of the closure path of the vertical current is needed. In the two-dimensional model, the closure path or electric current circuit is confined to the magnetic meridional plane, while the three-dimensional model allows the flow of the discharging current partly through a circuit in the magnetic east-west plane. But the discharging current in the east-west plane is smaller by a few orders of magnitude than that in the magnetic meridional plane because the longitudinal conductivity, which is effective only in the magnetic meridional plane, is a few orders of magnitude larger than the other conductivities which are effective in the magnetic east-west plane. Hence, the neglect of the discharging current in the east-west plane does not significantly

alter the values of the vertical current, the effective polarisation field  $E_p'$  and the east-west current density  $j_w$  generated by shearing winds.

The two-dimensional currents and electric fields resulting from local winds in the equatorial electrojet can be mapped out readily using the equations (14), (15), (16) and (20). The simplicity of this method is to be contrasted with the very elaborate numerical analysis of the differential equations which is needed in the conventional approach. Using a computer, we can obtain the numerical solutions of complicated differential equations. The main advantage of the present method consists of its ability to provide a clear insight into the complex physical processes, through a straightforward physical interpretation of the mathematical expressions for the relevant parameters like the vertical current, the potential drop  $e_r$  and so on. However, elaborate numerical analysis of the appropriate equations have to be used in dealing with more complicated situations like three dimensional currents and electric fields.

In the present method, the vertical current is calculated from equation (14) and the resultant polarisation electric field  $E_p'$  is

obtained from equations (15) and (16). The east-west current is calculated from equation (20). The east-west drift of the ionisation can be calculated using the value of  $E_p'$ , while the vertical drift of isolation can be calculated in terms of the  $(\mathbf{J}_w \times \mathbf{B})$  force.

Finally, a similar equivalent circuit formulation—with the same simplicity of the mathematical equations and the same advantage of straightforward physical interpretation—can be used in case of the equatorial electrojet generated by the east-west electrostatic field  $E_y$ . Such formulation is particularly useful when the electrostatic field  $E_y$  varies with latitude (and in altitudes as well whenever significant longitudinal variations are present on a comparatively short scale).

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## Vital effect on stable-isotope composition seen in foraminifera and coral skeletons

Jonathan Erez

Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

*Stable-isotope composition of carbonate precipitated by hermatypic corals and associated benthonic foraminifera is strongly influenced by photosynthesis of symbiotic algae. In situ  $^{14}\text{C}$  and  $^{45}\text{Ca}$  uptake experiments show that when photosynthesis increases, more light metabolic  $\text{CO}_2$  is incorporated in the skeleton. This mechanism explains the non-equilibrium isotopic compositions previously reported for these organisms (as well as for planktonic foraminifera), and have important implications for palaeoenvironmental determinations based on stable-isotope analysis.*

conditions. These observations conflict with the isotopic equilibrium assumption of the stable-isotope palaeoceanographic method, and can impose severe limitations on the interpretation of stable isotope data. Parker<sup>11</sup> suggested that symbiotic algae, existing in many species of planktonic and benthonic foraminifera<sup>12</sup>, may influence the isotopic composition of the skeleton. Recently, Grazzini<sup>9</sup> noted that planktonic foraminifera collected in the photic zone (possibly containing symbionts as indicated by their coloured protoplasm) deviate from equilibrium more than foraminifera with 'milky' protoplasm collected below the photic zone. But the effect of algal symbiosis on the stable-isotope composition of foraminiferal skeleton was never directly studied.

The relationship between  $\text{CaCO}_3$ -depositing organisms and their symbiotic algae are best demonstrated by hermatypic corals. Radioactive tracer experiments show that algal photosynthesis enhances coral calcification<sup>13</sup> and that some metabolic carbon is incorporated into the skeleton<sup>13,14</sup>. Hermatypic corals show specific and individual fractionation of stable isotopes as well as light non-equilibrium isotopic compositions<sup>15,16</sup>, again indicating incorporation of isotopically light metabolic  $\text{CO}_2$  into the skeleton<sup>13–16</sup>. Weber and Woodhead<sup>16</sup> suggested a model emphasising the importance of the symbiotic algae in determining the amount of light metabolic  $\text{CO}_2$  incorporated in the skeleton of hermatypic corals. This model, however, was not tested rigorously, and does not seem compatible with some recent observations<sup>17</sup>.

In order to study more directly the effect of symbiotic algae on the stable-isotope composition of foraminifera and corals, a field experiment was conducted in the Gulf of Eilat, Israel, during the summer 1975. The field and laboratory procedures are described elsewhere<sup>18</sup>, but briefly, hermatypic corals and benthonic foraminifera were double labelled *in situ* using  $^{45}\text{Ca}$  and  $^{14}\text{C}$  as tracers to measure calcification and photosynthesis. Dark and dead controls were run with the illuminated

STABLE isotopes of oxygen and carbon in foraminiferal skeletons are widely used in palaeoenvironmental studies. A basic assumption in these studies is that skeletal  $\text{CaCO}_3$  is deposited at or close to isotopic equilibrium with seawater; any biogenic fractionation that may occur is assumed to be constant, and this can be accounted for. Under this assumption, depth habitat for various planktonic species, palaeotemperatures, ice-volume estimates and palaeoclimatological trends for the Pleistocene have been calculated<sup>1–6</sup>. Foraminifera used in these studies are usually taken from sediment samples. However, when foraminifera from plankton tows were analysed the  $^{18}\text{O}/^{16}\text{O}$  ratios (measured as ‰ deviation from a standard  $-\delta^{18}\text{O}$ ) were often lighter (that is, enriched in  $^{16}\text{O}$ ) than the expected equilibrium values, by 0.5 to 1.5 ‰ (refs 7–9). Light non-equilibrium values of up to 2–3 ‰ for oxygen and carbon isotopes were also observed in shallow benthonic foraminifera<sup>10</sup>. It was pointed out<sup>7–10</sup> that different species of foraminifera as well as individuals within a species population can have different isotopic compositions, although they live in the same water and are exposed to the same physico-chemical



experiments in order to evaluate the effect of photosynthesis on calcification and the non-biogenic incorporation of radionuclides. Experiments used different species at the same depth and the same species at different depths. Radioactive analysis of a sample yielded three fractions: (1)  $^{45}\text{Ca}$  incorporated in the skeleton; (2)  $^{14}\text{C}$  incorporated in the skeleton (both measure calcification); and (3)  $^{14}\text{C}$  incorporated into the soft tissue. This fraction was obtained by dissolving the skeleton and retaining the insoluble residue on a filter, and represents mostly photosynthetically fixed carbon.

Radionuclides were measured by liquid scintillation. Counting efficiencies were 80% and 90% for  $^{14}\text{C}$  and  $^{45}\text{Ca}$ , respectively. Photosynthetic rate is reported in  $\mu\text{g C per mg IR per h}$  (IR is insoluble residue), and calcification rate is  $\mu\text{g Ca per g CaCO}_3$  per h. Precision of the analytical method (determined on five replicates) was  $\pm 3.6\%$  for photosynthesis and  $\pm 17\%$  of the reported value ( $1\sigma$ ) for calcification. Stable isotope analysis of oxygen and carbon in skeletal subsamples was carried out using commonly accepted techniques<sup>19</sup>. Before acid reaction, samples were combusted in a low-temperature oxygen-plasma furnace. This procedure removes organic matter without changing the skeletal isotopic composition. (W. Deuser, personal communication). Results are reported in ‰ relative to the PDB standard.

$$\delta^{18}\text{O} = \left( \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} - 1 \right) \times 1,000$$

Precision for both isotopes is better than  $\pm 0.1\%$ , obtained by continuous runs of standards, as well as replicates of many samples.

## Rates of photosynthesis and calcification

Rates of photosynthesis by coral symbionts (Table 1) range from 0.546 to 2.320  $\mu\text{g C per mg IR per h}$ , and are similar to those reported by Goreau<sup>19</sup> for Jamaican corals. These rates are on average 60 times higher than the dark controls and 300 times higher than the dead controls. Photosynthetic rates in the

foraminifer *Amphistegina lobifera* range from 1.058 to 1.997  $\mu\text{g C per mg IR per h}$  (Table 1); on average these rates are 35 times higher than the dark controls.

Rates of calcification of corals based on  $^{45}\text{Ca}$  uptake range from 25 to 1,212  $\mu\text{g Ca per g CaCO}_3$  per h. These values correspond to  $\text{CaCO}_3$  accretion rates of 0.006% and 0.304% per h, respectively (average 0.097% per h), and are similar to those reported by Goreau<sup>18</sup> for Jamaican corals. Drew<sup>20</sup> reported rates of calcification for corals in the Gulf of Eilat when exposed to adverse conditions, that are an order of magnitude lower (see ref. 18 for detailed discussion). Calcification in the foraminifer *Amphistegina lobifera* range from 938 to 3,481  $\mu\text{g Ca per g CaCO}_3$  per h. The average calcification rate of this species, which is a major constituent in the reef sediments<sup>21</sup>, is five times higher than the average calcification rate of the fastest growing coral measured (*Stylophora pistillata*). For both corals and foraminifera there is a strong enhancement of calcification in illuminated experiments compared to dark controls. Light/dark calcification ratios average  $\sim 11$  for corals and  $\sim 50$  for foraminifera. Calcification rates in dead controls are only half of those in dark controls. The consistent differences between illuminated experiments and the controls demonstrate the accuracy of the radiotracer method used in this study. The enhancement of skeleton deposition for corals and foraminifera in the light indicates that the symbiotic algae play an important role in this process. This confirms earlier *in situ* observations for corals<sup>13</sup>, and laboratory studies on benthonic foraminifera<sup>22,23</sup>.

In the depth profiles (Fig. 1) corals and foraminifera show mid-water photosynthesis and calcification maxima. Over-illumination in surface water may cause inhibition of photosynthesis as found in marine phytoplankton<sup>24,25</sup>. It seems that the symbiotic algae within the corals and foraminifera behave in a similar way. Because photosynthesis enhances calcification, mid-water calcification maxima are also observed (Fig. 1). It should be noted, however, that the positive correlation between photosynthesis and calcification for a single species at different depths (Fig. 1) does not exist when different species at the same depth are compared (Table 1). This may indicate that the calcification/photosynthesis ratio for a given species is constant

**Table 1** *In situ* radiotracer experiments and stable-isotope composition for corals and foraminifera in the Gulf of Eilat, Israel

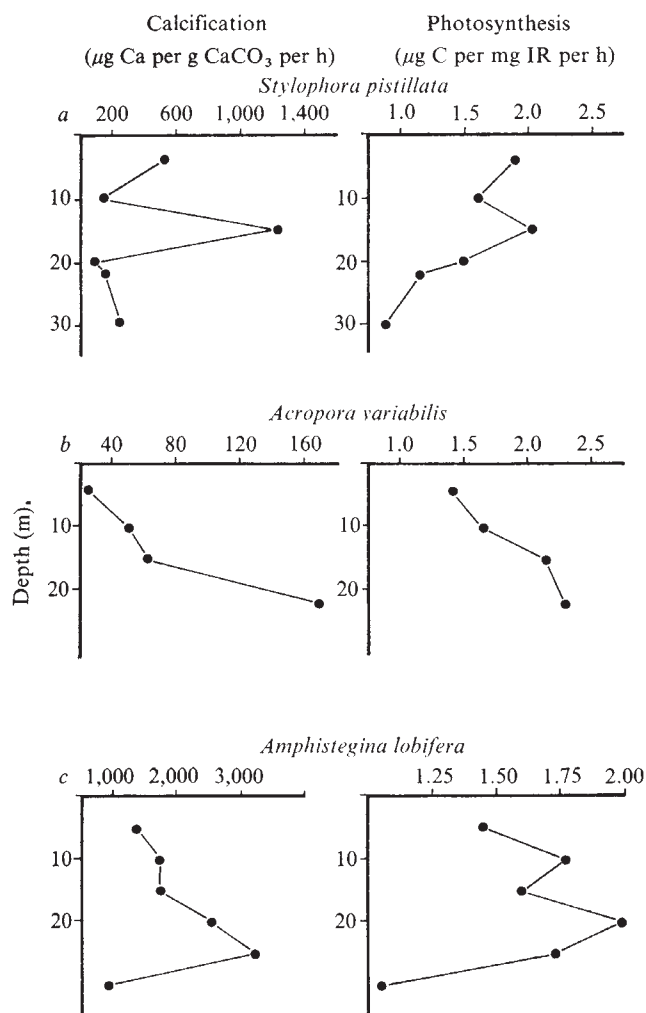
| Species                | Location  | Depth (m) | Photosynthesis<br>( $\mu\text{g C per mg IR per h}$ ) |       |        | Calcification<br>( $\mu\text{g Ca per g CaCO}_3$ per h) |      |      | $^{14}\text{C}/^{45}\text{Ca}$ Accretion rate ratio |       | $\delta^{18}\text{O}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|------------------------|-----------|-----------|-------------------------------------------------------|-------|--------|---------------------------------------------------------|------|------|-----------------------------------------------------|-------|---------------------------|---------------------------|
|                        |           |           | Light                                                 | Dark  | Dead   | Light                                                   | Dark | Dead | Light                                               | Dark  |                           |                           |
| <i>S. pistillata</i> 1 | Taba      | 4.5       | 0.826                                                 | 0.085 | 0.002  | 327                                                     | 8    | 4    | 0.172                                               | 1.192 | -1.82                     | -0.91                     |
| <i>S. pistillata</i> 2 | Taba      | 4.5       | 0.909                                                 | 0.028 | 0.008  | 208                                                     | 23   | 6    | 0.321                                               | 0.450 | -1.68                     | -1.22                     |
| <i>M. dichotoma</i>    | Taba      | 4.5       | 0.546                                                 | 0.006 | 0.002  | 129                                                     | 19   | 16   | 0.555                                               | 0.945 | +0.69                     | +1.58                     |
| <i>P. danae</i>        | Taba      | 4.5       | 1.707                                                 | 0.011 | 0.0002 | 118                                                     | 12   | 4    | 0.081                                               | 0.185 | -2.32                     | -3.49                     |
| <i>A. eurystoma</i>    | Taba      | 4.5       | 0.986                                                 | 0.013 | 0.004  | 70                                                      | 9    | 5    | 0.397                                               | 0.538 | -2.20                     | -1.81                     |
| <i>S. pistillata</i>   | MBL       | 4         | 1.93                                                  | 0.003 |        | 508                                                     | 4    |      | 0.128                                               | 0.281 | -2.18                     | -1.45                     |
| <i>S. pistillata</i>   | MBL       | 10        | 1.60                                                  | 0.001 | 0.005  | 124                                                     | 4    | 2    | 0.229                                               | 0.786 | -1.81                     | -1.12                     |
| <i>S. pistillata</i>   | MBL       | 15        | 2.04                                                  |       | 0.0004 | 1,216                                                   |      | 3    | 0.073                                               |       | -1.84                     | -1.68                     |
| <i>S. pistillata</i>   | MBL       | 22        | 1.16                                                  | 0.005 | 0.002  | 146                                                     | 6    | 3    | 0.243                                               | 1.014 | -1.82                     | -1.57                     |
| <i>S. pistillata</i>   | Ras Burka | 20        | 1.48                                                  | 0.010 |        | 85                                                      | 17   | 5    | 0.471                                               | 1.523 | -1.57                     | -0.71                     |
| <i>S. pistillata</i>   | Ras Burka | 30        | 0.90                                                  | 0.008 |        | 242                                                     | 21   |      | 0.104                                               | 1.290 | -2.10                     | -1.55                     |
| <i>A. variabilis</i>   | MBL       | 4         | 1.42                                                  |       |        | 25                                                      |      |      | 0.100                                               |       | -1.17                     | -1.69                     |
| <i>A. variabilis</i>   | MBL       | 10        | 1.65                                                  |       |        | 51                                                      |      | 2    | 0.344                                               |       | -1.46                     | -0.11                     |
| <i>A. variabilis</i>   | MBL       | 15        | 2.16                                                  | 0.012 | 0      | 62                                                      | 6    | 7    | 0.351                                               | 0.777 | -2.13                     | -2.38                     |
| <i>A. variabilis</i>   | MBL       | 22        | 2.32                                                  |       | 0.006  | 170                                                     |      |      | 0.256                                               |       | -2.38                     | -2.18                     |
| <i>A. lobifera</i>     | Taba      | 5         | 1.466                                                 | 0.036 |        | 1,375                                                   | 33   |      | 0.162                                               | 0.625 | -0.77                     | +1.12                     |
| <i>A. lobifera</i>     | Taba      | 10        | 1.799                                                 | 0.045 |        | 1,737                                                   | 36   |      | 0.187                                               | 0.222 | -0.70                     | +1.35                     |
| <i>A. lobifera</i>     | Taba      | 15        | 1.597                                                 | 0.052 |        | 1,743                                                   | 62   |      | 0.323                                               | 0.200 | -0.63                     | +1.27                     |
| <i>A. lobifera</i>     | Taba      | 20        | 1.997                                                 | 0.048 |        | 2,539                                                   | 32   |      | 0.192                                               | 0.375 | -0.54                     | +1.24                     |
| <i>A. lobifera</i>     | Taba      | 25        | 1.738                                                 | 0.052 |        | 3,481                                                   |      |      |                                                     |       | -0.56                     | +1.36                     |
| <i>A. lobifera</i>     | Taba      | 30        | 1.058                                                 |       |        | 938                                                     |      |      |                                                     |       | -0.54                     | +1.13                     |

Note the significant and consistent differences between experiments carried out in the light and the dark and dead controls.

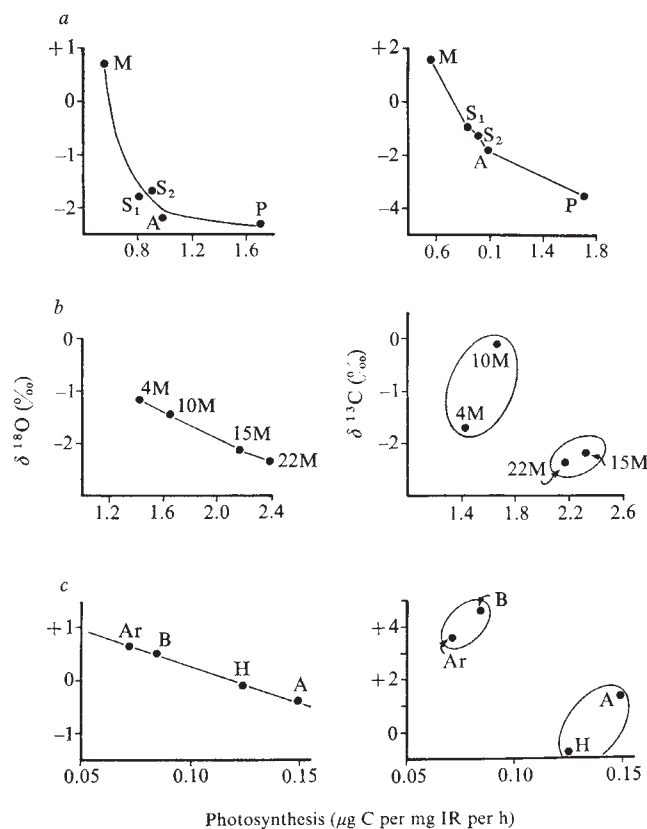
and that different species have different calcification/photosynthesis ratios.

### Stable-isotope composition

The stable-isotope results are shown in Table 1 and Fig. 2.  $\delta^{18}\text{O}$  range from  $-2.38\text{‰}$  to  $+0.69\text{‰}$  and  $\delta^{13}\text{C}$  from  $-3.49\text{‰}$  to  $+4.65\text{‰}$ . Equilibrium value for carbonate oxygen in the Gulf of Eilat is about  $+0.6\text{‰}$ , based on the Epstein *et al.*<sup>25</sup> equation, water  $\delta^{18}\text{O}$  of  $+2.0\text{‰}$  (Nissenbaum, unpublished data) and average temperature of  $22.9^\circ\text{C}$  (ref. 27). Almost all samples are much lighter than the expected equilibrium value for oxygen. The large range in values of  $\delta^{13}\text{C}$  indicates that many of the corals and the foraminifera are also out of equilibrium with respect to carbon isotopes. Seasonal variations and different depth habitats cannot explain the large variation in isotopic composition between and within species. Thus, it seems that most of the variability is caused by a 'vital effect'<sup>28</sup> involving metabolic activity in skeleton deposition. A clear trend can be observed (Fig. 2): the isotopic composition becomes lighter when the rate of photosynthesis increases. This is observed for both elements (oxygen and carbon), for different species at the same depth (corals and foraminifera) and for one species



**Fig. 1** Calcification and photosynthesis depth profiles for corals (a, b) and foraminifera (c), based on *in-situ* radiotracer experiments in the Gulf of Eilat, Israel. The reduction of photosynthesis in shallow water is possibly caused by photoinhibition, and may result in mid-water photosynthetic maxima. Because calcification is strongly enhanced by photosynthesis, mid-water calcification maxima are also observed.



**Fig. 2** Stable-isotope composition of the skeletons against photosynthesis of symbiotic algae in corals and foraminifera in the Gulf of Eilat, Israel. a, Different coral species at the same depth (4.5 m). M, *Millepora dichotoma* (a hydrozoan); S, *Stylophora pistillata*; A, *Acropora variabilis*; P, *Pocillopora danae*. b, One coral species (*Acropora variabilis*) at different depths. c, Different foraminifera species at one depth ( $\sim 10$  m). A, *Amphistegina lobifera*; Ar, *Amphisorus hemprichii*; H, *Heterostigina* sp.; B, *Borelis* sp. When photosynthesis increases, the isotopic composition becomes lighter. This indicates that as a result of photosynthesis by symbionts, more light metabolic  $\text{CO}_2$  is incorporated in the skeleton of these organisms.

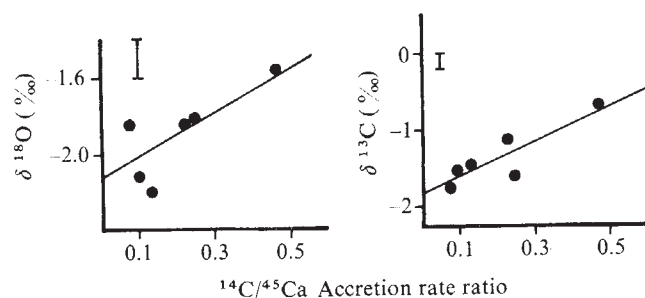
(*Acropora variabilis*) at different depths. Although there is considerable change in photosynthetic rates of *A. lobifera* in the depth range studied, there is relatively very small change in the isotopic composition of this species (Table 1). The reasons for this have yet to be studied; however, the average  $\delta^{18}\text{O}$  for this species is  $1.2\text{‰}$  lighter than the expected equilibrium value. Isotopic composition and rates of calcification do not correlate well (Table 1). Therefore it seems that skeletal growth rate *per se* is not the prime factor controlling the isotopic composition, as was concluded also by Weber *et al.*<sup>29</sup>.

### Metabolic $\text{CO}_2$ in the skeleton and symbiotic algae

Based on the light isotopic compositions of coral skeletons several authors suggested that some metabolic  $\text{CO}_2$  is incorporated in the skeleton<sup>13-16</sup>. The present study supplies independent evidence to support this idea based on calcification rates: because double labelling was used, calcification rates can be measured by  $^{45}\text{Ca}$  or  $^{14}\text{C}$  uptake into the skeleton. Ideally these rates should be equal; however, accretion rates based on  $^{45}\text{Ca}$  uptake are always 5 to 10 times higher than those based on  $^{14}\text{C}$  uptake (Table 1). As a result, the  $^{14}\text{C}/^{45}\text{Ca}$  accretion rate ratio is usually between 0.1 and 0.5. This phenomenon was first observed by Goreau<sup>13</sup> who interpreted it as a dilution of the  $^{14}\text{C}$  tracer by metabolic carbon pool, which is available in the



coral tissue for skeletogenesis. My observations strongly support Goreau's idea. The correlations with the isotopic compositions (Fig. 3) further suggest that the dilution is indeed by isotopically light metabolic  $\text{CO}_2$ . In dark experiments, the  $^{14}\text{C}/^{45}\text{Ca}$  accretion rate ratio is almost always higher than in the illuminated experiments, and in many experiments the ratio approaches one (Table 1), which indicates no relative dilution of the tracer. This suggests that photosynthesis increases the amount of metabolic  $\text{CO}_2$  in the skeleton, and independently supports the observation that when photosynthesis increases, the isotopic composition of the skeleton becomes lighter (Fig. 2).



**Fig. 3** The ratio between calcification rates based on  $^{14}\text{C}$  and  $^{45}\text{Ca}$  incorporation in the skeleton as opposed to the stable isotope composition in the coral *S. pistillata*. Ideally this ratio should be 1.0, but the ratio is lower than expected, indicating dilution of the  $^{14}\text{C}$  tracer by metabolic  $\text{CO}_2$ . When dilution by metabolic  $\text{CO}_2$  increases, the isotopic composition becomes lighter. The error bars represent  $2\sigma$  of the stable-isotope analysis.

Weber and Woodhead<sup>18</sup> proposed that the isotopic composition of coral skeletons is a mixture of isotopically heavy seawater bicarbonate and isotopically light metabolic (or respiratory)  $\text{CO}_2$ . They assumed that symbiotic algae utilise metabolic  $\text{CO}_2$  for photosynthesis and therefore predicted that when photosynthesis increases, less metabolic  $\text{CO}_2$  is available for incorporation in the skeleton, which consequently will become heavier. This prediction does not agree with the observations presented here (Fig. 2) that higher rates of photosynthesis are associated with isotopically lighter skeletons. Weber and Woodhead's model is based on numerous observations that corals have lighter isotopic composition at 18 m than at the surface, and on the assumption that photosynthesis is maximal at the surface and decreases with depth. The depth profiles presented here (Fig. 1) as well as the profile of Barnes and Taylor<sup>30</sup> show that photosynthesis is maximal at some mid-depth (15–25 m) rather than at the surface. Thus it is possible that the lighter isotopic composition observed by Weber *et al.* around 18 m is associated with higher photosynthetic rates, in agreement with the relationship between photosynthesis and isotopic composition presented here (Fig. 2). Recently, Weber *et al.*<sup>20</sup> measured the isotopic composition of the coral *Montastrea annularis* in the Caribbean at different depths. They found a minimum of  $\delta^{13}\text{C}$  at 18.3 m and interpreted this as support for their model. In view of the present results this profile can be reinterpreted to represent a mid-water photosynthetic maximum which is supported by other investigators<sup>17,18,30</sup>.

## Conclusions and implications

I conclude that skeletal carbonate of hermatypic corals and foraminifera is composed of a mixture of two components: (1) seawater bicarbonate that may be incorporated into the skeleton by an isotopic equilibrium process; and (2) metabolic  $\text{CO}_2$  that is enriched in  $^{12}\text{C}$  and  $^{18}\text{O}$ . The symbiotic algae, when active, enhance the overall metabolic activity of the host-algae complex, and increase the amount of metabolic  $\text{CO}_2$  in the organisms' internal  $\text{CO}_2$  pool. This will increase the share of metabolic  $\text{CO}_2$  component in the skeleton, which consequently

will become isotopically lighter. This model can explain the non-equilibrium compositions and the individual and specific fractionations that were reported for corals and shallow benthonic foraminifera<sup>10,16</sup>. Because many shallow planktonic foraminifera contain symbiotic algae<sup>12,31</sup>, the same model may be applied for these organisms to explain the non-equilibrium compositions and the specific fractionations they exhibit<sup>7–9</sup>.

Palaeoenvironmental interpretations (especially palaeotemperatures) based on the stable-isotope composition of planktonic foraminifera that contain symbiotic algae should be made with great caution. Isotopically light skeletons, for example, can represent change in isotopic composition of seawater or elevation of temperature (as was interpreted before), but can also represent an increase in the photosynthetic rate of the symbiotic algae.

This is well demonstrated by the depth profile for *A. variabilis* (Table 1, Fig. 2). A temperature gradient for this profile can be calculated, assuming that the  $\delta^{18}\text{O}$  against temperature curve for this species is parallel to the palaeotemperature curve of Epstein *et al.*<sup>26</sup>, as suggested by Weber and Woodhead<sup>16</sup>. The 'isotopic' temperature thus calculated at 22 m is 5 °C warmer than at 4 m. Such an inverted thermocline is obviously erroneous in the high energy reef environment of the Gulf of Eilat, where temperature is known to decrease slightly with depth<sup>27,32</sup>. This erroneous result is caused by increase of photosynthesis in this depth interval (Fig. 2). The role of metabolic  $\text{CO}_2$  from sources other than photosynthesis (such as food and respiration) may be of equal importance in producing a 'vital effect' on skeletal isotopic composition of organisms, as suggested by Goreau<sup>33</sup>. This may also apply to organisms that do not have symbiotic algae (for example, deep planktonic and benthonic foraminifera). To accurately calculate palaeotemperatures, depth habitats, ice-volume estimates, or other environmental parameters based on stable-isotope composition of biogenic carbonates, the 'vital effect' should first be studied and accounted for.

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# Nucleotide sequence and secondary structure of potato spindle tuber viroid

Hans J. Gross, Horst Domdey, Christine Lossow, Peter Jank, Manfred Raba & Heidemarie Alberty

Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG

Heinz L. Sänger

Justus-Liebig-Universität, Arbeitsgruppe Pflanzenvirologie, D-6300 Giessen, FRG

*The viroid of the potato spindle tuber disease (PSTV) is a covalently closed ring of 359 ribonucleotides. As a result of intramolecular base pairing, a serial arrangement of double-helical sections and internal loops form a unique rod-like secondary structure. PSTV is the first pathogen of a eukaryotic organism for which the complete molecular structure has been established.*

VIROIDS are unconventional infectious agents, causing diseases in certain higher plants similar to those caused by plant viruses. However, in contrast to conventional viruses and their infectious nucleic acids, viroids are minimal infectious agents. They exist as RNA molecules of molecular weight of about 120,000, free of coat protein. Economically important plant diseases are caused by the potato spindle tuber viroid (PSTV)<sup>1</sup>, the citrus exocortis viroid (CEV)<sup>2,3</sup>, the chrysanthemum stunt viroid (CSV)<sup>4,5</sup>, the cucumber pale fruit viroid (CPFV)<sup>6</sup>, the chrysanthemum chlorotic mottle viroid (CCMV)<sup>7</sup>, the coconut cadang-cadang viroid (CCCV)<sup>8,9</sup> and the hop stunt viroid (HSV)<sup>10</sup>. The mechanism of viroid replication and pathogenicity has not been established. Several possible viroid structures have been proposed, for example, single-stranded hairpins or double-stranded molecules with incomplete base pairing<sup>11-13</sup>. We showed recently that our viroid preparations contain at least 99% single-stranded, covalently closed circular RNA molecules<sup>14</sup>. In their native state they exist as rod-like structures with an axial ratio of 1:20, displaying thermal stability and high cooperativity of thermal denaturation<sup>15</sup>. Circularity, the common feature of viroids, was later confirmed by an electron microscopic study of another group<sup>16</sup>. Their samples, however, consisted mainly of linear molecules of heterogeneous size distribution<sup>17</sup>. The availability of homogeneous viroid preparations<sup>14,18</sup> has enabled us to determine the primary structure of PSTV as propagated in tomato, its experimental host. This sequence analysis leads to an unequivocal demonstration of viroid circularity and furthermore allows a unique secondary structure for PSTV to be proposed.

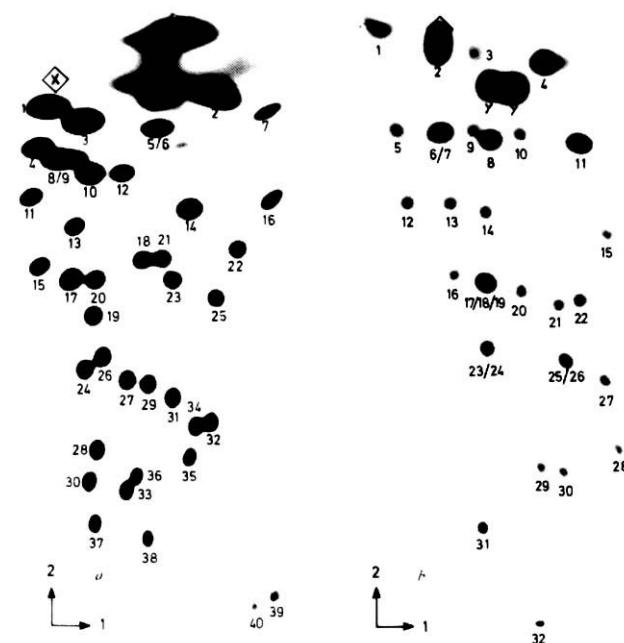
## Nucleotide sequence

Because of the minute amount of viroid present in infected plant material, radioactive labelling is essential for establishing the nucleotide sequence. Although it is possible to grow viroid-infected plants in nutrient solutions containing <sup>32</sup>P-orthophosphate<sup>19</sup>, the specific radioactivity of the isolated viroid is far too low for sequencing work because of the comparatively slow rate of replication. The RNA had therefore to be labelled *in vitro* after extraction, using [ $\gamma$ -<sup>32</sup>P]ATP and T4 phage-induced 5'-polynucleotide kinase from *Escherichia coli*<sup>20</sup>, although this method has not been used before for sequencing an RNA of this size. We had applied this technique previously in sequence

studies of various nucleic acids<sup>21,22</sup> and we have also used it to compare viroids of different origin on the basis of their homochromatography fingerprints<sup>18</sup>. The high resolution achieved in these viroid fingerprints allowed the separation of the complete RNase T<sub>1</sub> and RNase A fragments and their sequence analysis. In addition, new rapid techniques for sequencing long [5'-<sup>32</sup>P]-labelled RNA<sup>23,24</sup>, based on the same principles as the DNA sequencing procedure of Maxam and Gilbert<sup>25</sup>, were applied.

The RNase T<sub>1</sub> and RNase A fingerprints as shown in Fig. 1 clearly demonstrate the purity of our PSTV preparation. The oligonucleotide sequences from these fingerprints are shown in Table 1. From these data, the nucleotide

**Fig. 1** Autoradiograms of [5'-<sup>32</sup>P]-labelled oligonucleotides obtained from RNase T<sub>1</sub> (a) and RNase A (b) digests of PSTV. First dimension: ionophoresis on cellulose acetate strips, pH 3.5 (ref. 49); second dimension: homochromatography in 30 mM KOH 'homomix', pH 4.7 (refs 18, 50) on DEAE-cellulose thin-layer plates. The isolate of PSTV, originally provided by Dr T. O. Diener in the tomato (*Lycopersicon esculentum*) cultivar Rutgers, was propagated in the bush tomato *Rentita*. Viroids were purified from total nucleic acids extracted from systemically infected leaf tissue by a combination of conventional methods including fractionation with NaCl and cetyltrimethylammonium bromide followed by three preparative polyacrylamide electrophoreses<sup>14,18</sup>. Digestions and *in vitro* 5'-labelling with [ $\gamma$ -<sup>32</sup>P]ATP and T4 polynucleotide kinase were done exactly as described<sup>18</sup>. Sequences are listed in Table 1. Y, Unidentified nucleoside 3',5'-diphosphates. X, Position of the xylene cyanol marker. In (b) the xylene cyanol dye coincides with oligonucleotide 2.





composition of PSTV is 73×AMP (20.3%), 77×UMP (21.4%), 101×GMP (28.1%) and 108×CMP (30.1%). Overlapping fragments were obtained by controlled digestion of PSTV with enzymes followed by [5'-<sup>32</sup>P] phosphorylation and two-dimensional polyacrylamide gel electrophoresis<sup>26,27</sup>. The nucleotide sequences of fragments up to 20 nucleotides long were established by controlled nuclease P<sub>1</sub> degradation and two-dimensional homochromatography. This 'mobility shift' method was also applied to long PSTV fragments, whereby only the sequences of the 15–20 nucleotides from the 5' end were determined (Fig. 2a). The sequences up to 80 nucleotides from the 5' end were elucidated by additional

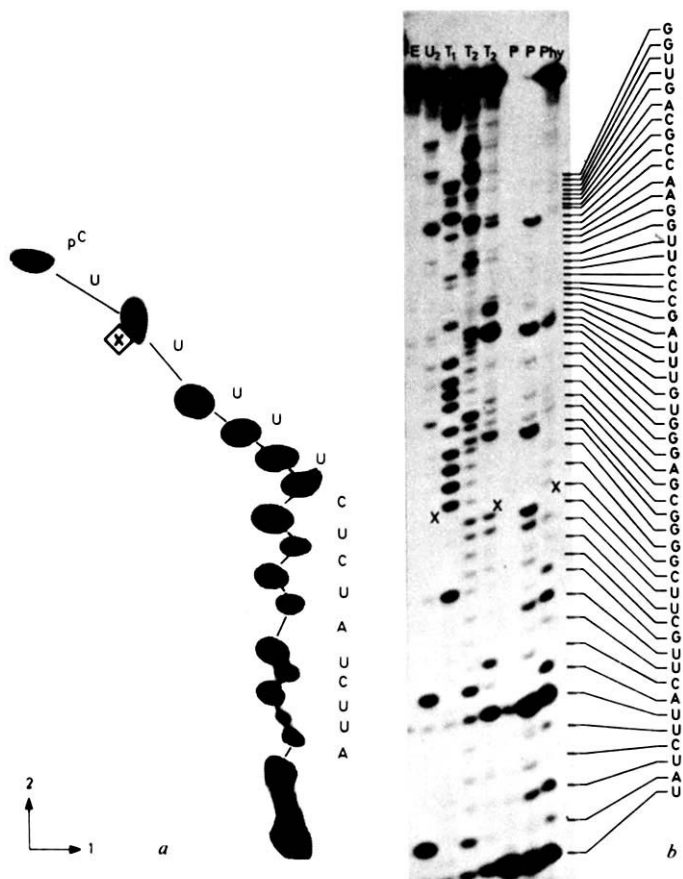
**Table 1** Sequence of labelled oligonucleotides from RNase digests

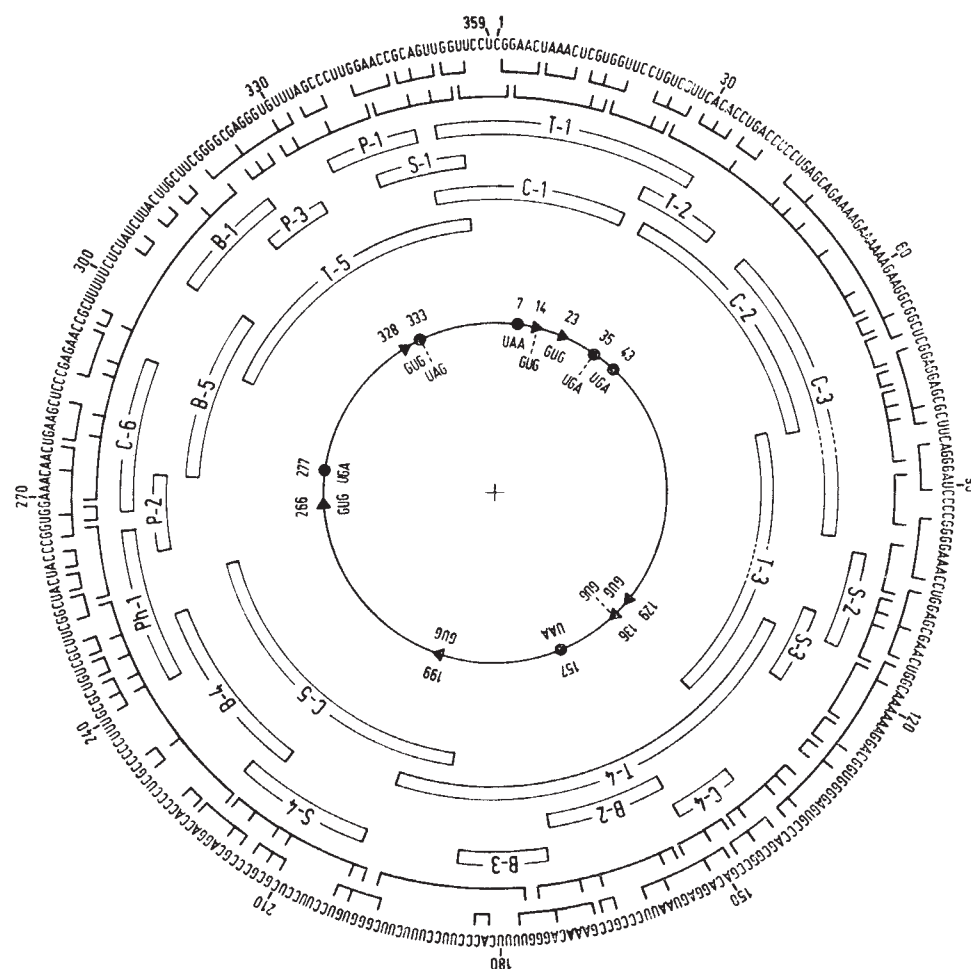
| spot | A                                       | a   | b | c |
|------|-----------------------------------------|-----|---|---|
| t-1  | C-G                                     | 6.4 | 6 | 7 |
| t-2  | U-G                                     | 7.3 | 7 | 7 |
| t-3  | A-G                                     | 8.0 | 8 | 8 |
| t-4  | C-C-G                                   | 2.0 | 2 | 2 |
| t-5  | C-U-G                                   | 1.2 | 1 | 1 |
| t-6  | U-C-G                                   | 1.2 | 1 | 1 |
| t-7  | U-U-G                                   | 1.0 | 1 | 1 |
| t-8  | C-A-G                                   | 2.7 | 3 | 3 |
| t-9  | A-C-G                                   | 0.9 | 1 | 1 |
| t-10 | A-A-G                                   | 2.1 | 2 | 2 |
| t-11 | C-C-C-G                                 | 1.2 | 1 | 1 |
| t-12 | C-U-C-G                                 | 1.1 | 1 | 1 |
| t-13 | A-C-A-G                                 | 0.9 | 1 | 1 |
| t-14 | C-U-U-C-G                               | 2.2 | 2 | 2 |
| t-15 | C-C-C-A-G                               | 0.8 | 1 | 1 |
| t-16 | U-U-U-A-G                               | 1.1 | 1 | 1 |
| t-17 | A-A-C-C-G                               | 1.7 | 2 | 2 |
| t-18 | A-A-C-U-G                               | 0.8 | 1 | 1 |
| t-19 | A-A-A-A-G                               | 1.0 | 1 | 1 |
| t-20 | C-U-C-C-C-G                             | 1.0 | 1 | 1 |
| t-21 | C-C-C-U-U-G                             | 0.7 | 1 | 1 |
| t-22 | U-U-C-C-U-G                             | 0.9 | 1 | 1 |
| t-23 | C-U-U-C-A-G                             | 0.9 | 1 | 1 |
| t-24 | A-A-A-C-A-G                             | 0.8 | 1 | 1 |
| t-25 | U-U-C-C-U-C-G                           | 0.7 | 1 | 1 |
| t-26 | A-U-C-C-C-C-G                           | 0.8 | 1 | 1 |
| t-27 | A-A-A-C-C-U-G                           | 1.0 | 1 | 1 |
| t-28 | A-A-A-A-A-G                             | 1.1 | 1 | 1 |
| t-29 | A-C-C-U-C-C-U-G                         | 0.8 | 1 | 1 |
| t-30 | C-A-A-A-A-A-G                           | 1.0 | 1 | 1 |
| t-31 | C-C-C-C-C-U-U-G                         | 0.9 | 1 | 1 |
| t-32 | U-A-A-U-U-C-C-C-G                       | 0.8 | 1 | 1 |
| t-33 | A-A-A-C-A-A-C-U-G                       | 0.9 | 1 | 1 |
| t-34 | U-C-C-U-U-U-C-C-U-G                     | 0.8 | 1 | 1 |
| t-35 | C-U-C-A-C-A-C-C-U-G                     | 0.7 | 1 | 1 |
| t-36 | C-U-A-C-U-A-C-C-C-G                     | 0.7 | 1 | 1 |
| t-37 | A-C-C-A-C-C-C-C-U-G                     | 0.8 | 1 | 1 |
| t-38 | A-A-C-U-A-A-A-C-U-G                     | 0.6 | 1 | 1 |
| t-39 | C-U-U-U-U-U-C-U-A-U-C-U-U-A-C-U-U-G     | 0.7 | 1 | 1 |
| t-40 | U-U-U-U-C-A-C-C-C-U-U-C-C-U-U-C-U-U-C-G | 0.6 | 1 | 1 |

| spot | B                                     | a    | b  | c  |
|------|---------------------------------------|------|----|----|
| A-1  | A-C                                   | 6.7  | 7  | 7  |
| A-2  | G-C                                   | 13.1 | 13 | 13 |
| A-3  | A-U                                   | 1.0  | 1  | 1  |
| A-4  | G-U                                   | 5.9  | 6  | 5  |
| A-5  | A-A-C                                 | 0.9  | 1  | 1  |
| A-6  | A-G-C                                 | 1.7  | 2  | 2  |
| A-7  | G-A-C                                 | 2.0  | 2  | 2  |
| A-8  | G-G-C                                 | 3.1  | 4  | 4  |
| A-9  | A-A-U                                 | 1.0  | 1  | 1  |
| A-10 | A-G-U                                 | 1.4  | 1  | 1  |
| A-11 | G-G-U                                 | 4.2  | 5  | 5  |
| A-12 | A-A-A-C                               | 1.0  | 1  | 1  |
| A-13 | G-A-A-C                               | 0.8  | 1  | 1  |
| A-14 | G-A-G-C                               | 0.8  | 1  | 1  |
| A-15 | G-G-G-U                               | 0.6  | 1  | 1  |
| A-16 | G-A-A-A-C                             | 0.7  | 1  | 1  |
| A-17 | A-G-G-A-C                             | 0.7  | 1  | 1  |
| A-18 | G-A-A-G-C                             | 0.5  | 1  | 1  |
| A-19 | G-G-A-A-C                             | 1.4  | 2  | 2  |
| A-20 | G-G-A-G-C                             | 0.7  | 1  | 1  |
| A-21 | G-G-G-G-C                             | 0.3  | 1  | 1  |
| A-22 | A-G-G-G-U                             | 0.6  | 1  | 1  |
| A-23 | G-A-G-A-A-C                           | 0.6  | 1  | 1  |
| A-24 | G-G-A-A-A-C                           | 0.6  | 1  | 1  |
| A-25 | A-G-G-A-G-U                           | 0.5  | 1  | 1  |
| A-26 | A-G-G-G-A-U                           | 0.5  | 1  | 1  |
| A-27 | G-A-G-G-G-U                           | 0.4  | 1  | 1  |
| A-28 | G-G-G-G-A-G-U                         | 0.2  | 1  | 1  |
| A-29 | G-G-G-G-A-A-A-C                       | 0.3  | 1  | 1  |
| A-30 | G-G-A-G-G-A-G-C                       | 0.2  | 1  | 1  |
| A-31 | A-A-A-A-A-A-G-G-A-C                   | 0.6  | 1  | 1  |
| A-32 | A-G-A-A-A-A-G-A-A-A-A-A-G-A-A-A-G-G-C | 0.1  | 1  | 1  |

Sequences of the [5'-<sup>32</sup>P]-labelled oligonucleotides from RNase T<sub>1</sub> (A) and RNase A (B) fingerprints of PSTV (Fig. 1) were determined by controlled nuclease P<sub>1</sub> digestion<sup>22,47,48</sup> followed by two-dimensional homochromatography (Fig. 2) and/or ionophoresis on DEAE-cellulose paper at pH 3.5 and pH 1.9. Uncorrected molar yields (a) were determined by Cerenkov counting of spots from several independent fingerprints. The molar yields of the oligonucleotides t-19 and A-12, respectively, were defined as 1.0, because short oligonucleotides with several adenosines at the 5' end are practically quantitatively phosphorylated. Low molar ratios, according to our experience, occur in the case of guanosine-rich and/or long oligonucleotides. Corrected molar yields (b) were obtained by cross-checking the oligonucleotide sequences from the RNase T<sub>1</sub> fingerprint with those from the RNase A fingerprint, and vice versa. Real molar yields (c) are derived from the final nucleotide sequence of PSTV (Fig. 3). The application of *in vitro* <sup>32</sup>P labelling in sequence studies of RNAs is summarised in refs 18, 22, 26 and 48.

**Fig. 2** Autoradiograms of the sequence analysis of a representative fragment (T-5) from a controlled RNase T<sub>1</sub> digest of PSTV. **a**, Two-dimensional homochromatography. A 10<sup>5</sup> c.p.m. aliquot of oligonucleotide T-5 plus 75 µg carrier tRNA in 10 µl 50 mM ammonium acetate, pH 5.3, was partially degraded with 5 ng nuclease P<sub>1</sub> at 50 °C. After 2, 5, and 10 min, aliquots were pipetted onto 1 µl 25 mM Na<sub>2</sub>EDTA, and heated to 100 °C for 4 min. Another aliquot was incubated for 2 h at 50 °C and used for thin-layer chromatographic 5' endgroup analysis. The partial digest was separated by two-dimensional homochromatography in a 15 mM KOH 'homomix'. **b**, Sequence analysis on polyacrylamide gel. Conditions for controlled enzymatic degradation: 1 to 5×10<sup>4</sup> c.p.m. of 5'-labelled oligonucleotide with 10 µg carrier tRNA were digested for 15 min at 50 °C in 10 µl 7 M urea, 1 mM Na<sub>2</sub>EDTA, 0.03% xylene cyanol and bromophenol blue, 20 mM sodium citrate, pH 5.0, with RNase U<sub>2</sub> (2 units), RNase T<sub>1</sub> (0.1 units), RNase T<sub>2</sub> (0.1 and 0.01 units), RNase A (2×10<sup>-6</sup> and 2×10<sup>-6</sup> units), respectively, and without enzyme (—E). Controlled digestion with RNase P<sub>1</sub> from *Physarum polycephalum*: 1 to 5×10<sup>4</sup> c.p.m. of 5'-labelled fragment with 20 µg carrier tRNA were digested for 15 min at 50 °C in 10 µl 4 mM Na<sub>2</sub>EDTA, 0.03% xylene cyanol and bromophenol blue, 150 mM sodium acetate, pH 4.5, with 3.5×10<sup>-3</sup> units RNase P<sub>1</sub>. This digest was pipetted onto 5 mg solid urea before loading it onto the sequencing gel. Electrophoresis was as described by Donis-Keller *et al.*<sup>23</sup>. Slots were 5 mm wide; X indicates the location of the xylene cyanol tracking dye. The location of fragment T-5 in the total sequence of PSTV is indicated in Fig. 3. Reading of the sequence of 60 nucleotides from the 5' end of T-5 starts from top **a** and continues, with an overlap of seven nucleotides, from bottom to top **b**.





**Fig. 3** Primary structure of PSTV. The nucleotides have been numbered clockwise from 1 to 359. Fragments from complete RNase A and RNase T<sub>1</sub> digestion are marked by brackets without numbers. A selection of overlapping sequences needed to establish the total primary structure of PSTV is presented as labelled bars. The dotted lines in fragments T-3 and C-3 indicate problems in reading parts of their sequences from polyacrylamide gels because of missing bands or abnormal distances between bands due to hairpin or dimer formation by these fragments. The bars indicate only the sequenced portions of the long overlapping fragments which were obtained by controlled nuclease digestions. B-1 to B-5: 6.5 µg PSTV and 0.4 µg bacterial alkaline phosphatase (Boehringer; containing an unknown nuclease<sup>22</sup>) were incubated in 8 µl 60 mM Tris-HCl, pH 8.0 for 1 h at 55 °C. The phosphatase was inactivated<sup>18</sup> before precipitation with 3 volumes of ethanol. C-1 to C-6: 10 µg PSTV were digested with 1 µg CM-RNase (modified ε-carboxymethyllysine-41 pancreatic RNase) in 10 µl 0.4 M NaCl, 50 mM triethylammonium bicarbonate (TEAB) buffer, pH 8.0, for 2 h at 20 °C. T-1 to T-5: 10 µg PSTV and 10<sup>-2</sup> U RNase T<sub>1</sub> were incubated in 18 µl 0.1 M TEAB, pH 8.0 for 2 h at 37 °C. P-1 to P-3: 10 µg PSTV and 20 ng RNase A were incubated in 34 µl 0.4 M NaCl, 60 mM TEAB, pH 8.0, for 2 h at 37 °C. The latter three digests were ethanol precipitated

and used without further inactivation of the RNases. S-1 to S-4: 10 µg PSTV and 0.2 U nuclease from *Staphylococcus aureus* were incubated in 20 µl 20 mM CaCl<sub>2</sub>, 100 mM sodium borate buffer, pH 8.4 for 45 min at 37 °C. The mixture was extracted four times with equal volumes of phenol, the oligonucleotides were then concentrated by ethanol precipitation. Ph-1: 5 µg PSTV and 14 mU RNase *PhyI* were incubated in 6 µl 0.5 M NaCl, 3 mM Na<sub>3</sub>EDTA, 70 mM sodium acetate buffer, pH 4.5 for 40 min at 50 °C, phenolised and precipitated as above. (5'-<sup>32</sup>P) phosphorylation of controlled digests was performed as described<sup>18</sup>. The fragments were separated by two-dimensional polyacrylamide electrophoresis exactly as described in refs 26 and 27. After autoradiography, the oligonucleotides were recovered from excised gel pieces by electroelution into dialysis bags and ethanol precipitation together with 50 µg carrier tRNA. The inserted solid circle indicates the location of possible initiator (▲) and terminator (●) codons in PSTV. Numbers refer to the first nucleotide of the corresponding triplet.

T-1 to T-5 are overlapping RNase T<sub>1</sub> fragments, whereas t-1 to t-40 (Table 1) are the complete RNase T<sub>1</sub> fragments of PSTV.

application of the rapid RNA gel sequencing technique (Fig. 2b) recently developed by Donis-Keller *et al.*<sup>23</sup> and Simonsits *et al.*<sup>24</sup>. This procedure involves polyacrylamide gel electrophoresis of controlled digests of long 5'-labelled RNA fragments produced with RNase U<sub>2</sub>, RNase T<sub>1</sub> and RNase A, respectively. By comparing the strong bands generated by these enzymes (Fig. 2b) the positions of the A, G and pyrimidine residues can be established. RNase T<sub>2</sub> digests provide the positions of all cleavable phosphodiester bonds. To distinguish the pyrimidines, Gupta and Randerath<sup>28</sup> and Simonsits *et al.*<sup>24</sup> applied RNase *PhyI* from *Physarum polycephalum*. This enzyme, which had been purified and characterised by Pilly *et al.*<sup>29</sup> does not cleave phosphodiester bonds at the 3' side of cytidines. We applied this enzyme and obtained similar results. Figure 2b shows such a sequence analysis of a representative PSTV fragment from a controlled RNase T<sub>1</sub> digestion. Thus the mobility shift and gel sequencing techniques (Fig. 2) complement each other and allow us in this case to establish the sequence of 60 nucleotides. However, we found that it was essential to have the sequences of the complete RNase T<sub>1</sub> and RNase A fragments (Table 1) established independently from the fingerprints (Fig. 1) by controlled nuclease P<sub>1</sub>

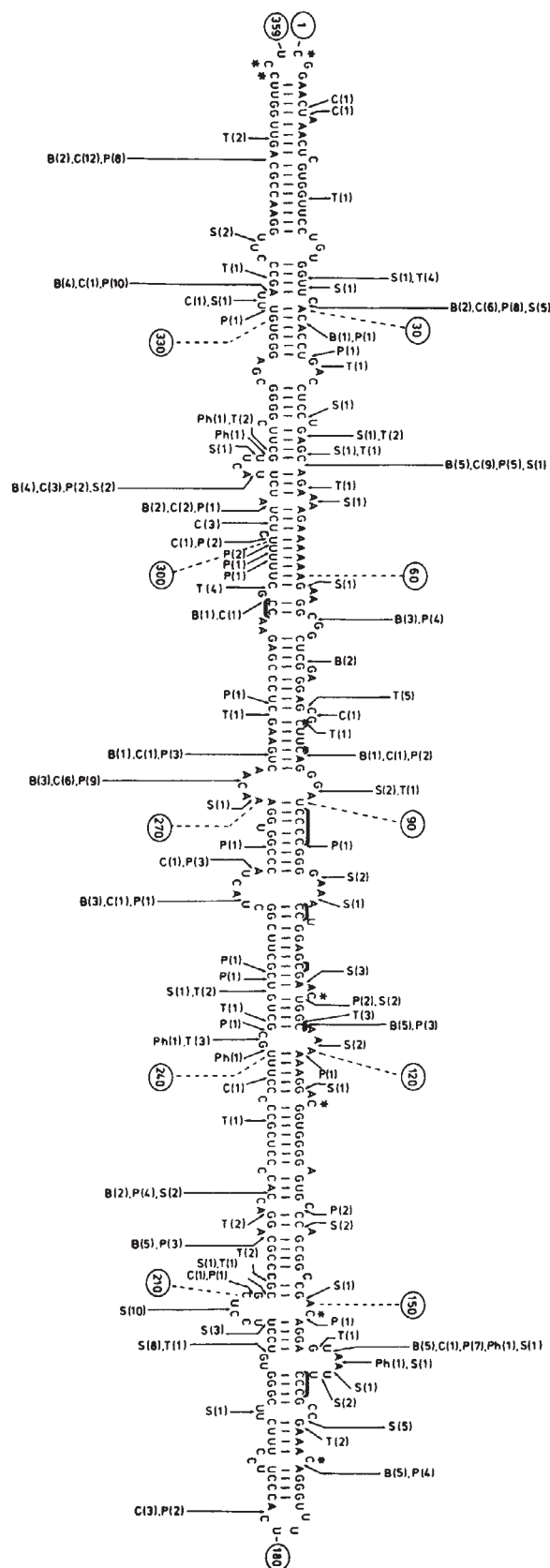
digestion and two-dimensional homochromatography. The knowledge of these sequences and of their molar ratios was necessary for establishing the full nucleotide sequence of PSTV. Difficulties in reading sequences directly from polyacrylamide gels were occasionally created by missing bands or by unusual distances between bands. This occurred in cases in which fragments had the capacity to form hairpins or dimers during enzymatic degradation and/or consecutive electrophoresis. This was observed especially for parts of the sequences of fragments C-3 and T-3 (Fig. 3). We have isolated several hundred long PSTV fragments generated by controlled digestion with various nucleases. From these fragments 165 were sequenced as described in Fig. 2 legend in order to establish the unique structure of PSTV with complete certainty.

In Fig. 3 a selection of 24 overlapping sequences has been arranged in such a way that both the sequence of the 359 nucleotides of PSTV and its circularity are unequivocally demonstrated.

## Secondary structure

The nucleotide sequence of PSTV was arranged in a secondary structure in such a way that a maximum number





**Fig. 4** Secondary structure of PSTV. This structure is derived from maximal intramolecular base pairing taking into consideration the observed nuclease and bisulphite sensitivity of PSTV. Arrows point to sites easily cleaved during controlled digestion; only exactly localised cleavage sites were considered. The length of the arrows is related to the number of splits observed at the respective site. Numbers at the arrows indicate how often we found cleavage; the corresponding enzymes are represented by letters as in Fig. 3. Reactivity of cytidine against bisulphite modification is marked by a heavy bar (resistance) or an asterisk (sensitivity, that is modification to uridine<sup>31</sup>).

of base pairs occurred. This crude model was then refined by considering (1) the location of cleavage sites deriving from the controlled enzymatic digestions of PSTV, and (2) the sensitivity of cytidines against bisulphite<sup>30</sup>, that is, resistance in double-stranded regions or modification to uridine in single-stranded loops<sup>31</sup>. This led to a model for the secondary structure of PSTV, presented in Fig. 4. The resulting extended molecule is characterised by a serial arrangement of double-helical sections and short internal loops. The two complementary halves of this covalently closed circle have virtually the same length—179 and 180 nucleotides, respectively. The sequence 1 to 179 consists of 50×AMP, 26×UMP, 59×GMP and 44×CMP, whereas the sequence 180 to 359 contains 23×AMP, 51×UMP, 42×GMP and 64×CMP. This results in 73 G : C, 38 A : U and 11 G : U pairs—a total of 122 base pairs. We are aware that alternative arrangements are possible for different sections of the molecule. We also have to consider the possibility that, on the other hand, the native secondary structure of PSTV need not necessarily contain the maximum possible number of base pairs. In Fig. 5 two such alternatives are shown which are thermodynamically more stable than the corresponding structures proposed in our complete model (Fig. 4). However, these arrangements do not seem to agree with the location of the enzymatic cleavage sites. There is little doubt that the rod-like secondary structure proposed here for PSTV agrees well with its thermal stability, the high cooperativity of the thermal denaturation process, the obvious lack of any indication for a particular tertiary structure and the axial ratio of 20 as observed for different viroids including PSTV<sup>14</sup>. In fact, an axial ratio of 23 is estimated for our model on the basis of X-ray crystallographic data from the A form of double-stranded RNA<sup>32</sup> if a helical structure equivalent to 180 base pairs is assumed. Moreover, our recent quantitative analysis of the thermal denaturation process of viroids provided independent evidence for the serial arrangement of double helical sections and internal loops in viroid molecules<sup>33</sup>. Therefore it does not seem necessary to discuss other possible and more globular viroid structures containing 80 to 90 base pairs. In conclusion, the native structure of PSTV as proposed in Figs 4 and 5—with an allowance for minor variations—seems self-evident. In nicked viroid molecules this secondary structure would certainly be maintained because of its high stability. The detailed thermodynamic discussion of these structures is in preparation.

## Features of interest

Considerable progress has been made in nucleic acid sequencing during the past few years and the primary structure of the nucleic acids of the bacterial viruses, RNA phage MS2<sup>34</sup> and DNA phage ΦX174 (ref. 35) have been established. These sequence determinations were based on the very efficient <sup>32</sup>P-labelling *in vivo* and on primed DNA synthesis *in vitro*, respectively. The RNA molecule we have sequenced is only one-tenth the size of MS2 RNA, but our work was hampered by the extremely low rate of replication of PSTV in plant tissue, and the small amounts of viroid RNA available. This made it necessary to apply a post-labelling technique. The problems inherent in this approach were overcome, because PSTV of the highest purity was available as well as a polynucleotide 5'-kinase preparation free of any trace of RNase activity<sup>18</sup>. At present PSTV is the largest RNA molecule the total nucleotide sequence of which has been worked out exclusively by an *in vitro* labelling technique. Moreover, PSTV is the first pathogen of a eukaryotic organism of which the complete molecular structure has been established.

The structure presented here raises many questions related to viroid replication and function. Modified nucleotides, a common feature of tRNAs, rRNAs and mRNAs have not been detected in 5' positions of any complete or

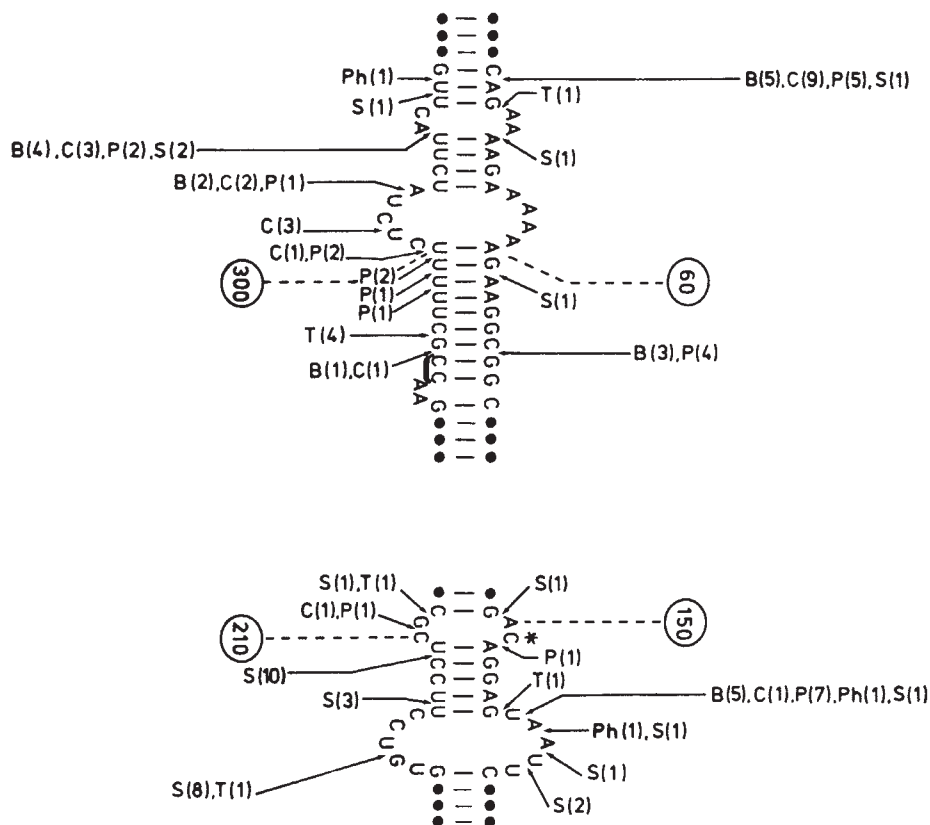


Fig. 5 Alternatives for the secondary structure of parts of PSTV. The sections shown here, especially that on the left, are thermodynamically more stable than the corresponding regions proposed in Fig. 4. However, the enzymatic cleavage sites do not seem to support these alternative structures (symbols as in Fig. 4). The location of enzymatic cleavage sites may not provide adequate information about the detailed secondary structure of such an uncommon RNA molecule.

partial PSTV fragment. Repeated efforts to introduce at least a single methyl group into PSTV (P.J. and H.J.G., unpublished), even in conditions which stimulate tRNA methylation<sup>36,37</sup>, with fractionated or unfractionated tRNA methylases from *E. coli*<sup>37</sup> or rat liver<sup>38</sup> were unsuccessful. However, we cannot yet exclude the presence of a few modified nucleotides in positions other than the 5' ends of the PSTV fragments investigated. Another feature of interest is a stretch of 18 purines, mainly adenosines, in positions 48 to 65 of PSTV. Similar sequences have also been detected in other viroids (H.J.G. *et al.*, unpublished) and they seem to be a common structural element of all viroids. Therefore, we propose to start counting the nucleotides in viroids in such a way that nucleotide 1 is located in the 5' terminal (left) loop of the purine-rich strand as done in Fig. 4. The main question arising in this context is whether viroids code for peptides or proteins. Previous attempts to translate PSTV and CEV into protein in several *in vitro* systems were unsuccessful<sup>39</sup>. Moreover, viroid RNA does not interfere with the *in vitro* translation of mRNA<sup>40</sup>. The circular arrangement of the uneven number of 359 nucleotides makes three rounds of translation possible in PSTV. The common AUG initiator triplet and several other codons are missing, but there are seven possible GUG initiator and six possible terminator triplets (inner circle in Fig. 3). The shortest possible translation product of PSTV would be a tetrapeptide, whereas the longest possible protein could result from more than two rounds of translation provided that in this case the weak terminator codon UGA translated. However, several aspects of the primary and secondary structure of PSTV seem to preclude its translation into peptides or proteins: ribosome binding sites (reviewed in ref. 22) at the 5' side of the GUG triplets are questionable and the 'cap' structure common to most eukaryotic mRNAs is missing. On the other hand there seems to be a strong evolutionary selection against CG neighbours in eukaryotic mRNAs<sup>41</sup>. Thus, the ratio of GC

to CG neighbours ranges from 10:1 in human<sup>42</sup> and rabbit<sup>43</sup>  $\beta$ -globin mRNA to 25:1 in the VP1 (ref. 44) and to 30:1 in the VP2 and VP3 mRNAs of SV40 (ref. 45). In contrast, the GC to CG ratio in PSTV is 1:1 (25:24 exactly). At the moment, therefore, it seems to be of little value to speculate that PSTV could be translated in chloroplasts, the prokaryotic<sup>46</sup> elements of the higher plant cell.

The general rule that infectious nucleic acids contain information for pathogen-specific proteins need not necessarily apply for viroids. If we assume this it follows that viroid replication and circularisation are completely dependent on host enzymes. The pathogenicity of viroids could result from a direct interaction with certain host components and/or metabolic pathways. There is little doubt that the viroid structure presented here (Fig. 4) contains as yet undiscovered recognition sites for replicating and ligating enzymes. Pathogenicity should also be reflected in this structure.

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# letters to nature

## Accelerating Universe revisited

THERE is growing evidence that the Hubble constant is of the order of  $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , rather than only half as great<sup>1</sup>. The purpose of this paper is to discuss the Friedman models that are consistent with such a large expansion rate, as well as with current estimates of stellar ages and the density of the Universe. If one accepts estimates of at least  $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$  for the Hubble constant, and that globular clusters have ages of the order of 16,000 Myr, then the only possible Friedman models of the Universe are those with a positive cosmological constant and  $q_0 < -1$ . Various independent tests of this conclusion are suggested.

The oldest globular clusters are currently assigned ages  $\geq 16,000 \text{ Myr}$  (refs 3, 4), so the dimensionless age  $H_0 t_0$  is apparently at least 1.6. Although the cluster ages, and the estimate of  $H_0 \sim 100$ , could conceivably be subject to errors of a factor of two, it is interesting to consider the implications of taking such a large dimensionless age at face value. To be specific, models with  $H_0 t_0 = 1.8$  will be discussed.

A value of  $H_0 t_0 > 1$  implies a present expansion rate that exceeds its past average value, so the Universe must be accelerating—that is,  $q_0 < 0$ —and it follows that the cosmological constant ( $\Lambda$ ) is positive. Lemaître's argument for  $\Lambda$  was of this form, and others have proposed a positive cosmological constant in various contexts<sup>5,6</sup>. Figure 1 of Gunn and Tinsley's<sup>6</sup> paper shows that models with  $H_0 t_0 = 1.8$  lie slightly to the right of the critical ( $\Lambda = \Lambda_c$ ) line and have  $q_0 < -1$ . Such models are of the Lemaître type, expanding from a singularity with deceleration up to an inflexion point (at redshift  $z_i$ ), then acceleration forever. (They are not extreme Lemaître models, with  $\Lambda/\Lambda_c - 1 \leq 1$  and with long quasi-static periods near  $z_i$ , as these have  $H_0 t_0 \gg 1$ .) The models with  $H_0 t_0 = 1.8$  have

positive curvature if the dimensionless density parameter  $\Omega$  exceeds 0.02, and they have an antipole (a redshift  $z_\pi$  at which the radial coordinate  $r = \pi$ ) if  $\Omega_0 > 0.08$ . It is well known that the slow-down at  $z_i$  and focusing effects near  $z_\pi$  can lead to peculiar observational properties (see for, for example, refs 5–11), as will be shown below.

Table 1 lists some properties of models with  $H_0 t_0 = 1.8$  and with  $\Omega_0$  in the range suggested by dynamical tests<sup>12–14</sup>. (Deuterium will be considered later.) Also listed are two models with  $\Lambda = 0$ , used below for comparison, although they are not consistent with the adopted dimensionless age. Table 1 gives the label (a letter and  $\Omega_0$ ) used below to refer to each model.

The Lemaître models have been chosen to agree with local cosmological data, so they are subject to global tests based on various types of lookback observations, discussed below.

According to various models for radio sources whose components are apparently separating faster than the speed of light<sup>15</sup>, one can derive single-step 'distances' to such sources, which lead to cosmological tests akin to the well-known relation between angular diameter and redshift. At small redshifts, the distances lead to values of  $H_0$ , and at greater redshifts,  $q_0$  can be determined. The various source models give different results, and here for illustration the light-echo model of Lynden-Bell<sup>2</sup> will be discussed.

Lynden-Bell<sup>2</sup> has already shown that nearby superluminal sources imply  $H_0 = 110 \pm 10 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , if his model is correct. The procedure for finding  $q_0$  (and an additional parameter,  $\Omega_0$  or  $\Lambda$ , at large enough redshifts) is as follows. With this model, data on superluminal radio sources give a measure of their expansion velocity,  $v_s$ , which is just  $c$  times a projection factor determined from the data. The rate of angular expansion,  $\chi$ , is also observed, and it is related to the velocity by  $\chi =$

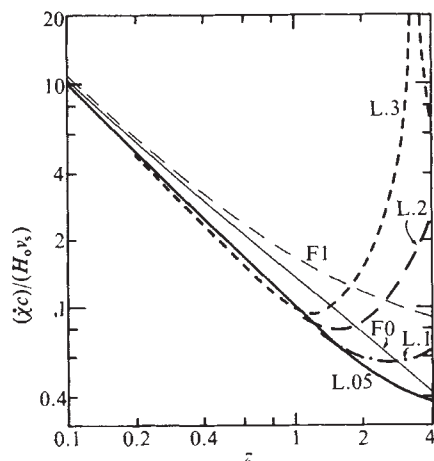


Fig. 1 The quantity determined by Lynden-Bell's interpretation of superluminal radio sources,  $(\chi c)/(H_0 v_s)$  is plotted as a function of redshift. The models are identified in Table 1.

$v_s/R_0 r$ , in standard notation. In a dimensionless form valid for models with or without  $\Lambda$ , the relation between  $\chi/v_s$  and redshift can be written,

$$(\chi c)/(H_0 v_s) = (1+z)/(H_0 D_L/c) \quad (1)$$

where  $D_L$  is the usual luminosity distance,  $R_0 r(1+z)$ .

Figure 1 illustrates this quantity for the models of Table 1, showing that a significant test for the Lemaitre models could be made using superluminal sources with a wide range of redshifts.

In first order, one finds a function of  $q_0$  (independent of  $\Lambda$ ):  $(\chi c)/(H_0 v_s) = z^{-1}[1 + (1+q_0)z/2 + O^2]$ , which is quite accurate at  $z \leq 0.5$ . The Lemaitre models considered here have values of  $q_0$  between  $-1.1$  and  $-1.5$ , so the test is predicted to yield  $q_0 < -1$  at redshifts of a few tenths, if indeed  $H_0 t_0 = 1.8$ . The sensitivity is such that the predicted value of  $\chi c/H_0 v_s$ , at  $z = 0.5$ , is at most 75% of that in the nearest model with  $\Lambda = 0$ .

At larger redshifts, the Lemaitre models have values of  $\chi/v_s$  that exceed the first-order estimates, by amounts that are very sensitive to  $\Omega_0$ . In particular, a sharp upturn should be found at  $z > 1.5$  if  $\Omega_0 > 0.2$ . (An extreme spike near  $z_\pi$ , like that shown for model L.3, may not be detected in a small sample of superluminal sources, since the co-moving volume near  $z_\pi$  is small; for example, in that model, only 10% of the co-moving volume between  $z = 1$  and  $z = 4$  lies at  $z > 2$ .) Thus the test may not only determine  $H_0$  and  $q_0$ , as Lynden-Bell noted, but may also set constraints on  $\Omega_0$ .

It should be noted that alternative models for the superluminal sources would give different values of  $H_0$  and  $q_0$  from the same data, so observational tests to choose among the models<sup>18</sup> are of great importance.

The angular diameter-redshift relationships for these models are given by a dimensionless quantity that is just  $(1+z)$  times the right-hand side of equation (1):  $\theta c/H_0 y = (1+z)^2/(H_0 D_L/c)$ , where  $y$  is the proper diameter. The extra redshift factor means that minima occur at smaller redshifts, and the Einstein-de Sitter model (labelled F1), which is monotonic in Fig. 1, has a shallow minimum at  $z = 5/4$ . Features corresponding to those discussed above could, in principle, be used to test the Lemaitre models: they have small values of  $\theta/y$ , appropriate to  $q_0 < -1$ , at  $z < 0.5$ , and upturns at greater redshifts, depending on  $\Omega_0$ . However, this test seems less promising than Lynden-Bell's, because of the large dispersion in proper sizes of radio sources, the difficulty of obtaining metric angular diameters of galaxies, and the likelihood of evolution of proper sizes on  $10^9$ -yr time

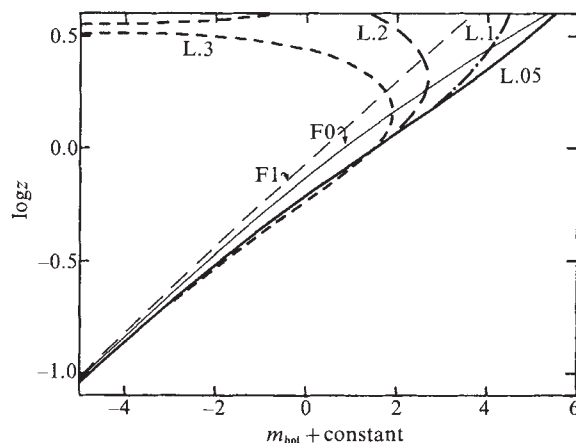


Fig. 2 Hubble diagrams for the models of Table 1. The abscissa is the apparent bolometric magnitude of an unevolving point source, normalised to  $5 \log z$  at small values of  $z$ .

scales. The great advantage of the former test is that the relevant intrinsic source property ( $v_s$ ) is determined directly in each case—provided that the model is valid, of course—so problems associated with dispersion and evolution do not arise.

Schematic Hubble diagrams are shown in Fig. 2, where the abscissa is the bolometric magnitude of a point-source standard candle, normalised to  $5 \log z$  at small  $z$ . Of course, real objects will have Hubble diagrams differing from these because of K terms, aperture effects, selection, and so on, but the schematic versions serve to illustrate the interesting points. Again, the diagram is closely related to Fig. 1, as

$$m_{bol} + \text{constant} = 5 \log(H_0 D_L/c).$$

At  $z < 0.5$ , the Hubble diagram again measures  $q_0$  independently of  $\Lambda$ , according to the first-order relation  $m_{bol} + \text{constant} = 5 \log z + 1.09(1-q_0)z + O^2$ . (Note that the 'constant' includes evolution of absolute magnitudes.) Galaxies should

Table 1 Properties of model universes with  $H_0 t_0 = 1.8$  or  $\Lambda = 0$

| Model label           |                        | L.05  | Lemaitre models |       |       | Fiducial ( $\Lambda = 0$ ) models |      |
|-----------------------|------------------------|-------|-----------------|-------|-------|-----------------------------------|------|
|                       |                        |       | L.1             | L.2   | L.3   | F0                                | F1   |
| Density               | $\Omega_0$             | 0.05  | 0.10            | 0.20  | 0.30  | 0.0                               | 1.0  |
| Deceleration          | $q_0$                  | -1.07 | -1.18           | -1.34 | -1.46 | 0.0                               | 0.5  |
| Age                   | $H_0 t_0$              | 1.80  | 1.80            | 1.80  | 1.80  | 1.0                               | 0.67 |
| Cosmological constant | $\Lambda/\Lambda_c$    | 6.70  | 2.41            | 1.51  | 1.31  | 0                                 | 0    |
|                       | $\Lambda/3H_0^2$       | 1.09  | 1.23            | 1.44  | 1.61  | 0                                 | 0    |
| Curvature             | $(kc^2)/(R_0^2 H_0^2)$ | 0.15  | 0.33            | 0.64  | 0.91  | -1.0                              | 0.0  |
| Inflexion             | $\{z_i/t_0$            | 2.52  | 1.90            | 1.43  | 1.21  | —                                 | —    |
|                       | $\{t_i/t_0$            | 0.30  | 0.35            | 0.39  | 0.41  | —                                 | —    |
| Antipole              | $\{z_\pi$              | —     | 24.6            | 5.66  | 3.42  | —                                 | —    |
|                       | $\{t_\pi/t_0$          | —     | 0.009           | 0.057 | 0.097 | —                                 | —    |



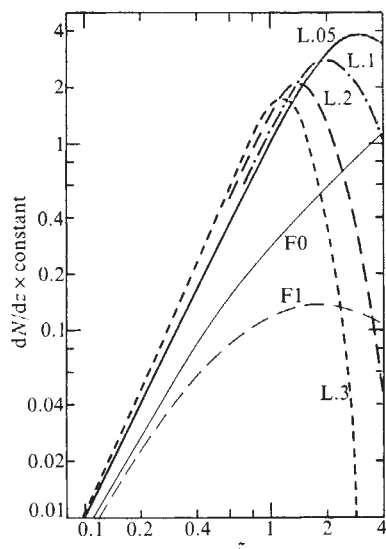


Fig. 3 Differential redshift distributions of objects with uniform coordinate density, in the models of Table 1. The unit of  $N$  is  $4\pi c^3/H_0^3$  times the local proper density.

therefore yield  $q_0 < -1$ , after correction for evolution, if the Lemaître models under consideration are valid. Unfortunately, this test is indeterminate, because of uncertainties in the evolutionary correction<sup>16</sup> and because of large discrepancies in the apparent value of  $q_0$ , before correction, obtained in different recent studies<sup>17,18</sup>. The Lemaître models are not inconsistent with some of the possibilities. For example, as Gunn and Tinsley<sup>6</sup> stressed, the true value of  $q_0$  must be negative if its apparent value is near zero<sup>17</sup> and if stellar evolution is more important than dynamical.

Quasars at high redshifts could be used to search for some of the extreme curvature effects in the Hubble diagram, but again there are practical and theoretical problems. For example, a vertical rise like that predicted for model L.2 near  $z = 2$  could be mimicked by a sample magnitude limit, especially in the presence of a large scatter. And the bright peaks near  $z_\pi$  could be missed because of the small co-moving volume in that region, or because  $z_\pi$  may occur before the epoch of quasar formation, or because of gravitational defocusing by intervening galaxies<sup>7</sup>.

The Lemaître models differ greatly from those with  $\Lambda = 0$  in their redshift distributions for objects with a constant coordinate density, as shown in Fig. 3. There are maxima at the inflexion redshifts,  $z_i$ , and deep minima at the antipoles,  $z_\pi$ . But unfortunately, redshift distributions are not directly observable because samples are always subject to some magnitude or flux limit. It is well known that the model-dependences of the  $N(z)$  and  $m(z)$  relationships cancel in first order, making the  $N(m)$  relationship very insensitive to  $q_0$ . The Lemaître models do have distinctive  $N(m)$  relations if one reaches a redshift where the Hubble diagram turns back to brighter magnitudes. The cumulative  $N(m)$  relationship steepens at that point (especially in a model like L.3, where its redshift almost coincides with  $z_i$ ), and then flattens abruptly because any fainter objects are beyond  $z_\pi$ , where  $dN/dz$  is very small.

Schmidt's<sup>19</sup> statistic  $\langle V/V_{\max} \rangle$  could be significantly affected by the peculiar  $N(z)$  relations of the Lemaître models, if the quantities  $V$  and  $V_{\max}$  were calculated for a model with  $\Lambda = 0$ . For example, the steep  $dN/dz$  in model L.05 would mimic density evolution in a standard model; and the sharp rise and fall of  $dN/dz$  in model L.3 could be mistaken for an increase of density with redshift out to a cutoff that is apparently due to reaching the formation redshift. (Such effects in extreme Lemaître models have been considered by Shklovsky<sup>10</sup> and Kardashev<sup>11</sup>, to account for the distribution of quasar

absorption redshifts at a time when most known values were very close to 1.95.) It would be difficult to draw any conclusions about the model from this test, of course, because a finite formation time and subsequent density evolution of quasars are very reasonable hypotheses. One can only note that  $V/V_{\max}$  distributions would have to be reinterpreted if one of the Lemaître models were validated.

A large value of the Hubble constant poses problems for cosmological production of deuterium<sup>6,12</sup>. For standard big bang conditions, the empirical deuterium abundance requires the present baryon density of the Universe to be less than  $4 \times 10^{-31} \text{ g cm}^{-3}$ , so that the contribution of baryons to  $\Omega_0$  must not exceed  $0.018(110/H_0)^2$ . But studies of the luminosity density and dynamics of galaxies suggest a total  $\Omega_0$  in the range 0.1–0.3 (refs 12–14). The dilemma could be solved by various modifications of the standard picture. For example, a large fraction of the total density could be due to heavy neutrinos (while baryons constitute only the visible matter of galaxies)<sup>20</sup>, or a greater baryon density would be allowed if the Universe has a non-zero lepton number<sup>21</sup>. The alternative of non-cosmological deuterium production has been widely studied, but no plausible sites have been found<sup>22</sup>.

If one takes seriously a value of  $H_0 \sim 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and globular cluster ages  $\sim 16 \times 10^9 \text{ yr}$ , then the only Friedman models that can describe the Universe are Lemaître models with  $\Lambda > 0$  and  $q_0 < -1$ . Properties of such models have been discussed in detail elsewhere<sup>5–9</sup>, and the emphasis here has been on possible tests for those with  $H_0 t_0 = 1.8$  and  $0.05 < \Omega_0 < 0.3$ . The best prospect would be an extension to large redshifts of Lynden-Bell's<sup>2</sup> test using superluminal radio sources, if indeed the geometry of his model applies to the actual mechanism of such sources; the results should yield  $q_0 < -1$ , corresponding to small values of  $\dot{x}/v_s$ , from sources at redshifts of a few tenths, with a levelling and possibly an increase in  $\dot{x}/v_s$  at greater redshifts.

In summary, if  $H_0$  is established to be as great as  $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , one is forced from two directions either into problems with astrophysical theory or into nonstandard cosmologies. (1) Either a non-cosmological source of deuterium must be found, or most of the density of the Universe is not in baryons, or other standard big bang conditions must somehow be modified. (2) Either the ages of globular clusters must be revised down by nearly a factor of two, or the Friedman models are invalid, or there is a positive cosmological constant.

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BEATRICE M. TINSLEY

Yale University Observatory,  
New Haven, Connecticut

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## Diffuse $\gamma$ -ray background from Seyfert galaxies

At least 20% of all Type I Seyfert galaxies are detectable<sup>1,2</sup> (at the comparable Ariel 5 and Uhuru sensitivities) as X-ray sources in the  $\sim 2$ –10 keV band. Both the Ariel 5 and Uhuru results indicate the X-ray luminosity function is quite steep ( $dN/dL \sim L^{-2}$ ) so that more sensitive HEAO X-ray detectors may reveal that nearly all Type I Seyfert galaxies are X-ray sources. It is suggested here that the diffuse background spectrum observed at hard X-ray and  $\gamma$ -ray energies can be accounted for by Seyferts, given their individual (hard) spectra.

As 4U1410–03 has been precisely located<sup>3</sup> as coincident with NGC5506, which may be a Type 2 Seyfert but morphologically resembles Cen A, the integrated X-ray emission from all emission line galaxies and active galaxies may exceed the  $\sim 15\%$  contribution previously estimated<sup>1,2</sup> for the diffuse X-ray background at  $\sim 2$ –10 keV. If these sources typically have photon spectra flatter than  $dN/dE \sim E^{-2.4}$ , which is<sup>4</sup> the approximate diffuse background above  $\sim 20$  keV, their contribution to the diffuse background spectrum at energies  $E > \bar{E}_c$  will become  $\sim 100\%$ . The critical energy

$$\bar{E}_c \approx [31.9]^{10} E^{-\alpha+1} dE]^{1/(2.4-\alpha)} \text{ keV}$$

is the energy at which the average Seyfert spectrum,  $dN/dE = AE^{-\alpha}$ , crosses the diffuse background spectrum. Thus to account for all of the high energy diffuse background flux, individual Seyferts and active galaxies must typically have high energy cutoffs with  $E_c \lesssim \bar{E}_c \approx 300$  keV–10 MeV for  $\alpha \sim 1.5$ –2.

Some recent observations suggest this may usually be true. The hard X-ray flux from the archetypical Type 1 X-ray Seyfert, NGC4151, has now been detected (with  $\alpha \sim 1.5$ ) through at least  $\sim 200$  keV and possibly several MeV (refs 5, 6). As NGC 4151 is relatively close and among the brightest X-ray Seyferts, its detection is reminiscent of the detection<sup>7</sup> of the nearby active galaxy Cen A with  $\alpha \sim 1.9$  at several MeV. Increased sensitivity would confirm whether these spectra are typical for more distant members of each class of object. In fact, the Type I Seyfert 3C120 (with  $z = 0.032$ ) has also been positively identified<sup>8</sup> as an X-ray source and is fit by a spectral index  $\alpha \sim 1.2$ –2.0. At least one much more distant ( $z \approx 0.047$ ) Seyfert has now also been identified<sup>9</sup> with 4U0241+61, which has<sup>10</sup> a very hard spectrum ( $\alpha \sim 1.0$ ) through  $\sim 1$  MeV and then steepens (with  $\alpha \sim 3$ ) at energies through  $\sim 100$  MeV, if its identification with the COS-B  $\gamma$ -ray source<sup>10</sup> CG135+1 is correct.

Thus in all cases where spectra have been measured for individual Seyferts and active galaxies, their spectra are both hard ( $\alpha \sim 1$ –2) and extend to cutoff energies ( $E_c \lesssim 100$  keV–1 MeV) large enough for their summed spectra to account for the diffuse background at energies  $\lesssim \bar{E}_c \sim 100$  keV. Where  $\gamma$ -ray spectra at  $E \sim 1$ –100 MeV are measured<sup>10,11</sup> or at least limited<sup>12</sup> (as for Cen A; C. Fichtel, personal communication) there is direct evidence for a high energy cutoff ( $E_c \sim 1$ –10 MeV) or steepening with  $\alpha \sim 3$ . This spectral shape can be explained by Compton–synchrotron models<sup>12</sup> where the typically flat spectrum of a self-absorbed synchrotron source in the galaxy nucleus is scattered into a self-similar spectrum in the X-ray– $\gamma$  ray range. The cut off is then due to the primary spectrum cut off in the infrared range where synchrotron lifetime effects are usually important, and variations (time scales  $\lesssim h$ –d) in the hard X-ray flux correlated with changes in the radio–infrared spectrum are expected<sup>12</sup>.

The diffuse background spectrum might then be expected to show a flattening at  $E \sim \bar{E}_c$ , where the Seyfert contribution is  $\sim 100\%$ , and a steepening at higher energies. The most recent summary<sup>13</sup> of low energy ( $\lesssim 1$  MeV)  $\gamma$ -ray data and high energy ( $\gtrsim 35$ –100 MeV) diffuse flux in fact show this shape, with a marginal flattening ( $\alpha < 2.4$ ) at  $\sim 1$ –10 MeV and  $\alpha \approx 2.85^{+0.5}_{-0.3}$  at  $E \lesssim 35$  MeV. As the contribution ( $\sim 15\%$ ) of Seyferts in the 2–10 keV range satisfies the limits for granularity of the background<sup>2</sup>, so also must their dominant contribution at high energies. Finally, if all galaxies have recurrent active phases or evolve through a Seyfert-like stage, then perhaps their typically hard spectra with a range of apparent cutoffs  $E_c > E_{c,\min} \approx 20$  keV could account for the entire diffuse flux with  $\alpha \approx 1.4$  ( $E \leq E_{c,\min}$ ) and  $\alpha \approx 2.4$  ( $E > E_{c,\min}$ ). However, whereas this is questionable due to uncertain evolutionary effects, the contribution now observed for (primarily) Type 1 Seyferts at least strongly suggests that Seyferts can account for all of the diffuse background at hard X-ray through  $\gamma$ -ray energies.

*Note added in proof:* Strong observational support for the Seyfert origin of the diffuse background is given by the recent announcement by Schonfelder at the Symposium on  $\gamma$ -ray Spectroscopy in Astrophysics (Goddard Space Flight Center, 28 April 1978) that  $\gamma$  rays have been detected from NGC4151. The spectrum seems to extend the power law from the hard X-ray range to a break at about 3 MeV, and is thus consistent with our prediction for the individual sources comprising the diffuse spectrum.

JONATHAN E. GRINDLAY

Harvard-Smithsonian Center for Astrophysics,  
60 Garden Street,  
Cambridge, Massachusetts 02138

Received 3 January; accepted 28 February 1978.

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## Coherently overturned flaps surrounding craters

MANY large impact and explosion craters exhibit coherently overturned flaps in the form of ejecta blankets<sup>1–7</sup> in which the pre-existing stratigraphy of the cratered ground is preserved in detail, but inverted. Sequential ejection of material into ballistic trajectories could produce a coarsely graded inversion of the material, but would not retain detailed stratigraphic coherence. The field evidence from fully excavated craters<sup>3–7</sup> shows that even totally incompetent strata composed of free flowing sand may be traced continuously through a hinge region below the crater rim outwards to at least three times the crater rim radius. The strata retain their integrity and relative position despite being reduced by stretching from an original thickness of some tens of centimetres to less than a centimetre at the extreme limit of coherent overturning. Figures 1 and 2, a photograph and a stratigraphic plot<sup>3</sup> of the hinge region of the Prairie Flat crater at the Defence Research Establishment, Suffield, illustrate the phenomenon. Similar examples are given in the reports of the excavation<sup>3–4</sup>. The effect was



present in all the Suffield trials, and was inconsistent with a free flight mechanism in which discrete elements of sand and clay would follow independent trajectories. However, in very small scale experiments in sand<sup>8</sup>, which would be most likely to exhibit a free flight phenomenon, the coherence in the overturned flap increased markedly when the sand was saturated with water, as compared to the case with dry sand. In this note I suggest that such coherent overturning may be produced by a wave of hydrodynamic type, which becomes a 'plunging breaker' of the type described by Longuet-Higgins and Cokelet<sup>9</sup>. Evidence to support this suggestion is given below, and consequences of the mode of formation are predicted and compared with the field data.

Seismic surface-wave motion, which exhibits prograde displacement orbits of hydrodynamic type, first detected in the vicinity of nuclear explosions, was described and called H-waves by Leet<sup>10</sup>. The suggestion that these waves were unique to explosive events is not now accepted, but it is certain that they are quite commonly produced by explosive sources. I<sup>11,12</sup> confirmed the presence of tilted prograde elliptic orbits in the surface waves near Suffield explosions which produced craters, but also detected orthogonally tilted retrograde elliptic orbits and classified the motion in terms of discrete branches of the Rayleigh-wave solution. In the case of surface explosions, it was demonstrated<sup>12</sup> that a modified type of Press-Ewing<sup>13</sup> coupling occurred between the ground and atmosphere, resulting in large amplitude surface waves of constant frequency. A more detailed computation of the dispersion curves for the test site was used by Hasegawa<sup>14</sup> to demonstrate the theoretical possibility of prograde motion consistent with the experimental data.

It has also been shown<sup>7</sup> by combining data on permanent displacement of marked elements of the ground<sup>15</sup> with

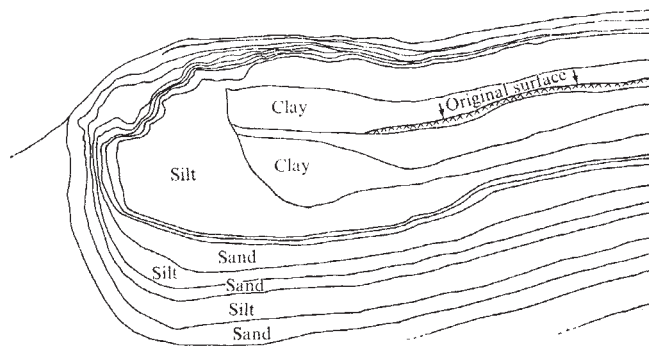


Fig. 2 Stratigraphy of prairie flat crater rim.

velocity gauge data<sup>16</sup> and the stratigraphy<sup>3</sup> of the Prairie Flat test site that a prograde elliptic motion was large enough to produce permanent displacement in the ground below the ejecta blanket. It is not certain that this interpreted displacement of material through three-quarters of a tilted prograde elliptic orbit<sup>7</sup> needs to be correlated with the elastic surface seismic waves detected beyond the plastic displacement zone, but it is probable.

According to Longuet-Higgins and Cokelet<sup>9</sup> the essential criterion for a breaking wave is that a wave of large amplitude suffers a rapid retardation of its forward motion, so that the front profile tends to steepen and overturn. These authors discuss the difference between a spilling breaker and a plunging breaker, and show that in the latter case a smooth jet of material is ejected from the top front face of the wave, and the flow in this jet may remain laminar even to the point where the jet impacts upon the 'undisturbed' surface ahead of the breaking wave. The calculations are detailed in ref. 9 together with computer generated cross-sections demonstrating the internal flow for the early part of the process. A more generalised account by Cokelet<sup>17</sup> describes the main features of breaking waves in water.

The experimental craters at Suffield were all formed in lacustrine deposits with large void ratios, saturated at depth but with much connate water even above the water table. Such a material is essentially thixotropic and behaves under stress more like a fluid than a solid. The geological significance of connate water has been described in ref. 18 and some of the consequences for crater mechanics in ref. 4. In naturally occurring impact craters where coherent overturning has been detected, the rigidity of the rock may resemble, on a scale basis, the much weaker material at the experimental site.

The potential for a wave of hydrodynamic type is thus demonstrated by the experimental data. It can also be shown that if such a wave is generated by the explosion, it will be retarded very rapidly. At the onset, the material will be displaced at a velocity close to that of the detonation wave in the explosive (say  $7 \text{ km s}^{-1}$ ). The shock wave induced in the ground by this motion attenuates rapidly and converts to a variety of seismic waves, the main surface manifestation being the surface seismic wave whose characteristics have already been described. At the Suffield test site the velocity of propagation of these elastic surface waves is in the region of  $1 \text{ km s}^{-1}$ , though due to the stratigraphy of the site, the medium is highly dispersive. An initially short pulse develops into long trains of surface waves which couple progressively<sup>12</sup> to the airblast. Thus a ground motion, originally of large amplitude and high velocity, decays into a small amplitude train of low velocity seismic waves in a distance comparable with the extreme limit of ejecta deposition. It is not yet possible to describe this decay in detail, but refs 7, 11, 12 give limited aspects of the process. For an impacting meteorite, the initial velocity will be related to that of the meteorite and may be far higher than that of the explosive detonation front.

Fig. 1 Rim section of prairie flat crater.



The foregoing indicates that a wave of hydrodynamic type may be formed, and may break in either the spilling or plunging mode. It remains to consider the probable consequences of such a mechanism and compare these with the experimental data.

First, consider the nature of the laminar jet ejected from the front face of a plunging breaker. As shown by the computed cross sections, such a jet will be in the form of a nappe (or recumbent anticlinal fold), so that when the jet impacts upon the surface it will superimpose the mobile strata in a folded form upon the undisturbed strata.

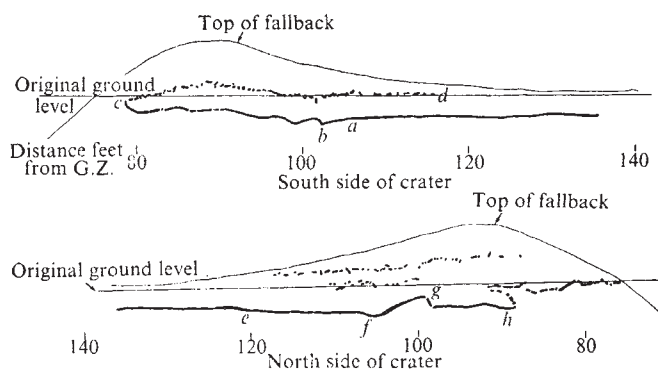


Fig. 3 Displacement of horizontal marker chain in 1961 Suffield 100-ton trial.

The field data cited so far only indicates two sets of strata, one the undisturbed bedding and the other the 'inverted' bedding perfectly preserved in the ejecta blanket. However, the upper elements of the ejecta blanket are subjected to late stage erosion and may become too turbulent for easy recognition of the original layering, especially when such an effect is not suspected. (Even the coherent overturning which is now easily detected in the field was not recognised in the majority of craters studied before the excavation of the Suffield craters.) Also, where any part of the upper layers of the ejecta blanket retain clear stratification with beds in the 'normal' attitude, the effect would probably be described as 'thrust faulting'. Thrust faulting is frequently reported from the relevant regions of large craters<sup>1-4</sup>, but usually from the rim and hinge regions where the stratification is most commonly exposed. One of the Suffield experiments, however, in which a 100-ton TNT charge was detonated on the surface (in 1961) incorporated a set of markers in a horizontal chain just below the original pre-cratering surface. Excavation of the crater revealed the markers disposed in the pattern shown in Fig. 3 (using the data of D. J. James). These data have been interpreted<sup>3,4</sup> as demonstrating coherent overturning and thrust faulting within the ejecta blanket. However, these data may also be interpreted as supporting the present hypothesis of emplacement of the ejecta blanket. The evidence of small recumbent folds is clear, and the triple layering evident in the north section may represent the preservation of a large recumbent fold rather than a thrust sheet.

Second, striations, caused by sliding contact of blocks in the ejecta blanket of the Ries crater, may be explained<sup>19</sup> by a form of roll-glide transport, suggesting a turbulent flow region of the ejecta blanket. Overturning and sliding of discrete surface blocks was also documented from the Suffield trials<sup>3</sup>. Such a mechanism is inherent in the second form of breaking wave—a spilling breaker<sup>8,16</sup>. In this form, the wave breaks by spilling forward from its crest a highly turbulent layer of material (visible as a froth in water waves) quite different from the laminar projection of a jet in the plunging type of breaker. Both types of breaking may occur in the cratering process, even at the same crater.

Attention is also drawn to the so-called 'wet craters' on Mars<sup>20</sup>. In these craters there is clear evidence that the ejecta blanket has flowed around pre-existing obstacles. This is consistent with the behaviour to be expected of a jet from a breaking wave but may also, of course, be explained by any other form of fluidised transportation of the material in the blanket. It seems inconsistent with ballistic emplacement unless all the material, including the last to impact, travelled in a relatively high velocity, low angle sheaf of trajectories.

Finally, if the breaking wave hypothesis is valid, one can anticipate that the ground will be depressed by the incipient trough ahead of the breaking wave. In the region of plastic deformation (or thixotropic behaviour) this downwarping may be preserved. The phenomenon is certainly present at all the craters excavated at Suffield, with the downwarping coinciding roughly with the region covered by the coherently overturned blanket. Various attempts have been made to correlate this downwarping with the inward movement of underlying material<sup>6</sup>, which is demonstrable in some cases<sup>4</sup> but there is insufficient resolution of the field data at present to indicate whether the downwarping was a consequence of, or the cause of, the flow. Certainly, downwarping implies either the removal or the compaction of underlying material.

Thus, the suggestion made here that the mechanism of deposition of coherently overturned ejecta blankets may be related to the formation of a hydrodynamic type of wave motion, which breaks in the plunging pattern, is consistent with the field data and is worthy of further investigation.

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G. H. S. JONES

Defence Research Board,  
Department of National Defence,  
101 Colonel By Drive,  
Ottawa, Ontario, Canada K1A 0K2

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## The extensional flow capillary as a new method for extensional viscosity measurement

THE behaviour of non-newtonian fluids in extensional flow is of both theoretical and practical importance. However, because of the difficulty of imposing a purely extensional deformation on a fluid material, very few experimental measurements of extensional viscosity have been obtained in well defined kinematic conditions. All of the experimental techniques currently available which utilise constant extension rate kinematics involve the application of a tensile stress to a cylindrical sample, and thus require the assumption of uniform deformation of



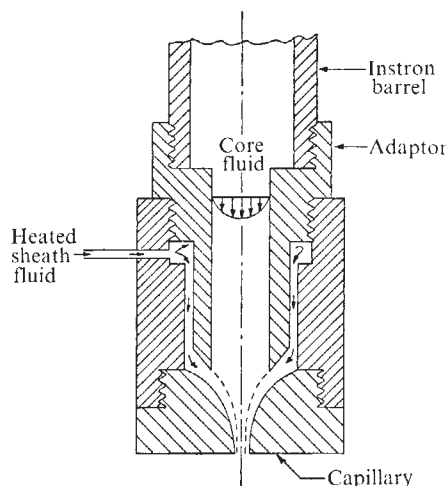


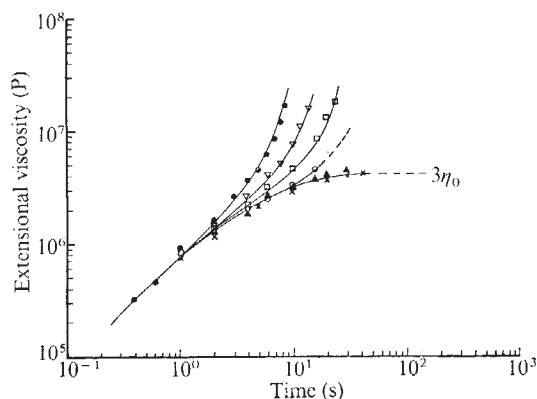
Fig. 1 Schematic diagram of the extensional flow capillary rheometer.

material which is bounded by a free surface. These techniques are applicable only for very high viscosity materials ( $> 10^5$  P), and are difficult to use in the study of materials whose resistance to extensional deformation is 'tension thinning' because of a tendency of the sample to neck and fail. We describe here an extensional flow capillary rheometer which we have evaluated for the measurement of extensional flow properties of a wide variety of materials over a broad range of extension rates. Recent discussion<sup>1</sup> of a 'speculative rheometer' of apparently similar design emphasises the value of such a device.

A schematic illustration of the extensional flow capillary rheometer is shown in Fig. 1. The basic design concept is the extrusion of a test material as the core component in a converging sheath-core flow field. The sheath component is a low viscosity material which continuously lubricates the capillary wall, and thus allows for plug flow or pure extensional deformation of the core material. A gear pump is used to meter the flow of sheath lubricant into the system, and an Instron capillary rheometer to measure the force required to maintain a constant extrusion rate of core material through the capillary.

The converging capillary is designed to maintain a constant extension rate in the core material. The ratio of the radial thickness of the sheath fluid to the radius of the core fluid is a very strong function of the capillary angle, and consequently the radius of the core fluid at any position in the capillary is not simply proportional to the radius of the capillary. As a

Fig. 2 Extensional viscosity versus time for polystyrene at 155 °C, for extensional rates ( $\dot{\epsilon}$ ) of 0.413 s<sup>-1</sup> (●), 0.260 s<sup>-1</sup> (▽), 0.165 s<sup>-1</sup> (□), 0.066 s<sup>-1</sup> (○), 0.0413 s<sup>-1</sup> (▲) and 0.026 (×).



result, the capillary design requires a solution of the converging sheath-core velocity field. In our work, this solution was approximately obtained through the assumption that the velocity field at any point in the capillary is given by the steady-state solution for stratified newtonian bicomponent flow in a cone with the cone angle equal to the angle of the local tangent to the capillary wall. Obviously, in the limit of zero sheath flow rate, the core radius becomes proportional to the capillary radius, and the capillary design is easily obtained from the specification of constant extension rate. However, this limit is not easily attained experimentally as the flow field in the capillary becomes increasingly unstable as the sheath-to-core flow-rate ratio is reduced.

In the operation of the extensional flow capillary rheometer, the sheath-to-core flow-rate ratio is generally held constant at

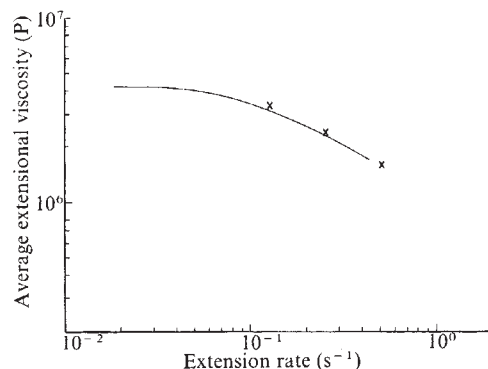


Fig. 3 Average extensional viscosity versus extension rate for polystyrene at 155 °C. Total strain, 1.68. The curve is calculated from experimental results given in Fig. 2. Crosses represent points derived by extensional flow capillary rheometer.

the value of 0.7, while the core flow rate varies over a wide range. In these conditions, the constant extension rate,  $\Gamma$ , in the core material is proportional to the core extrusion rate, and may be obtained directly from the Instron plunger speed. The tensile stress,  $T$ , acting on the core material is calculated from the measured Instron stress using the following equation:

$$T = [(F_{\text{tot}}/A_{\text{brl}}) - \Delta P_{\text{adtr}}]A_{\text{brl}} - F_{\text{back}}/A_0^{\text{core}} \quad (1)$$

Where,  $F_{\text{tot}}$  is the total force measured by the Instron load cell,  $A_{\text{brl}}$  is the cross-sectional area of the Instron barrel,  $\Delta P_{\text{adtr}}$  is the pressure drop in the adaptor section of the rheometer (see Fig. 1; this value is experimentally measured as a function of core flow rate before installation of the capillary),  $A_0^{\text{core}}$  is the initial core cross-sectional area ( $A_0^{\text{core}}$  = adaptor tube cross-sectional area), and  $F_{\text{back}}$  is the total back force exerted by the hydrostatic pressure in the sheath fluid on the core material in the capillary.  $F_{\text{back}}$  is calculated from the measured pressure in the sheath, assuming this material is incompressible and inviscid. Using the tensile stress,  $T$ , and the constant extension rate,  $\Gamma$ , the average extensional viscosity is calculated as

$$\eta_{\text{ext}} = T/\Gamma \quad (2)$$

The extensional viscosity results obtained from the extensional flow capillary rheometer are average viscosity values for the integrated total strain in the core material. In the operation of the rheometer, one obtains the average extensional viscosity as a function of extension rate under the condition of constant total strain. In contrast, most of the published extensional viscosity data have been obtained using equipment similar to that of Meissner<sup>2</sup>, in which one obtains point strain values of the extensional viscosity as a function of time of extension in conditions of constant extension rate. As we indicated

previously<sup>3</sup>, the Meissner representation of extensional viscosity can easily be replotted as a function of extension rate for constant values of total strain. Extensional viscosity results previously obtained<sup>4</sup> on polystyrene using a Meissner type apparatus are shown in Fig. 2. In order to compare these results with measurements from the extensional flow capillary rheometer, the data of Fig. 2 are replotted in Fig. 3 in the form of average extensional viscosity versus extension rate for the indicated constant total strain level. The average extensional viscosity values are obtained from the point strain values in the following way. For any given extension rate, the residence time of core material in the capillary is calculated as a function of capillary length  $z$ . Combining this calculation with the data of Fig. 2, the  $z$ -dependence of the extensional viscosity is obtained, and the average extensional viscosity,  $\eta_{\text{ext}}$ , is calculated as

$$\eta_{\text{ext}} = \int \eta_{\text{ext}} A(z) dz / \int A(z) dz \quad (3)$$

where  $A(z)$  is the  $z$ -dependence of the core cross-sectional area. Also included in Fig. 3 are experimental viscosity results obtained on the same polystyrene melt using the extensional flow capillary rheometer. Both sets of results are in close agreement, and thus lend support to the utility of the new technique for measurement of extensional viscosity.

The range of applicability of the new rheometer is quite extensive. So far we have successfully tested both polymer melts and solutions, some having zero shear-rate viscosities as low as  $10^3$  P, and have attained extension rates up to  $200 \text{ s}^{-1}$ . In general, the upper limit of extension rate for any given material is determined by a flow instability, which is manifested as an alternating extrusion of pure sheath and pure core material. This instability can be minimised, however, through the selection of appropriate sheath/core viscosity and flow rate ratios.

Details of the design and operation of the rheometer, and more extensive experimental results, will be presented elsewhere.

A. E. EVERAGE, Jr  
R. L. BALLMAN

Technical Center,  
Monsanto Textiles Company,  
Pensacola, Florida 32575

Received 5 December 1977; accepted 3 March 1978.

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## Durability of vitrified highly active waste from nuclear reprocessing

THE future of nuclear power is often said to depend on the development of a satisfactory method for dealing with highly radioactive waste such as the liquid which is produced during the extraction of plutonium from irradiated nuclear fuel. (See recommendation 27 in ref. 1). Much attention is now being given to developing processes suitable for solidifying highly active liquid waste<sup>2</sup> and conversion to a borosilicate glass seems the most favourable route. Here we report a recent investigation at Harwell which supports the belief that the installation of a satisfactory full-scale vitrification process will be successfully achieved.

In the early 1960s a pilot plant was built at Harwell to demonstrate the feasibility of operating a highly active vitrification process using industrial equipment. This was the Fingal process<sup>3</sup> which converted liquid waste into a 50 kg block of glass contained in a stainless steel cylinder

150 cm in length by 15 cm in diameter. The glass was made at  $1,050^\circ\text{C}$  by adding 43 parts of  $\text{SiO}_2$  and 32 parts of  $\text{Na}_2\text{B}_4\text{O}_7$  to 25 parts of the waste (calculated as oxides). The pilot plant programme was completed in early 1966 and by then it had been used in 47 runs of which eight used genuine (not simulated) highly active waste brought specially from Windscale.

Since then the blocks of radioactive glass in their stainless steel containers have been kept in a special store at Harwell. Recently, the most active of these blocks ( $\sim 14,000 \beta\gamma\text{-Ci}$  in 40 kg of glass) was examined to check the properties of the glass 11 yr after its manufacture. Using shielded cave facilities a sample of glass was removed from this block. First a hole was drilled through the 6 mm thick steel container and then a core of glass extracted along a diameter using a 19 mm i.d. diamond trepanning tool.

Perhaps the most important property of glass used to incorporate nuclear waste is its resistance to aqueous leaching. Of comparable importance is its physical and mechanical integrity, as any disintegration of the glass block will increase the surface available for leaching. The essential point is to ascertain whether either of these properties deteriorates over time due to devitrification or to radiation damage. Our results are reassuring in both respects.

A simple accelerated leach test was performed by placing a 150 mg sample of glass in a flask of distilled water for 7 d at  $90^\circ\text{C}$  and measuring its weight loss. The accuracy of this test is normally within  $\pm 15\%$ . (We usually quote leach rates calculated from weight losses measured after removal of the loosely adherent surface film that forms during leaching. However, this was not possible with this highly active sample. Removal of the film would have increased the apparent leach rate typically by 50%.) A similar but inactive reference glass was prepared, for comparison, using a simulated liquid waste and it was leached under identical conditions. Because the 11-yr-old Fingal glass exhibited some devitrification care was taken to crystallise the reference glass to the same extent, although separate tests showed that the degree of devitrification had little effect on the leach rate. It was found that the leach rate at  $90^\circ\text{C}$ , for highly active Fingal glass was  $0.33 \times 10^{-3} \text{ g cm}^{-2} \text{ d}^{-1}$ ; and for the equivalent non-active glass,  $0.35 \times 10^{-3} \text{ g cm}^{-2} \text{ d}^{-1}$ . This shows that after 11 yr there is no ageing phenomenon; the leach resistance of the glass is as good as the freshly prepared inactive version. (At ambient temperature the leach rate will be a factor of approximately 100 lower, corresponding to a linear rate of about 4 mm per 1,000 yr.)

Even if no disintegration within the steel container had occurred before sampling, the trepanning operating might have been expected to cause considerable shattering of the glass, a brittle solid. In fact, however, almost perfect cylindrical cores were obtained. Each core consisted of two or three large pieces each up to 7 cm long with completely intact cylindrical surfaces and just a minor amount of debris. It seems, therefore, that there has been no tendency for the glass block to disintegrate.

The specific activity of the Windscale waste from which the Fingal glass was made, was low by current standards. The fission product content of the waste was only 10% compared with the contemporary value of about 50%. Thus the radiation damage (whether by  $\beta\gamma$ - or more importantly by  $\alpha$ -radiation) suffered by the Fingal glass is low compared to that which future commercial vitrified waste will experience. However, more recent work has shown that  $\alpha$ -radiation doses in similar glasses equivalent to many thousands of years of exposure have negligible effect (within a factor of 2) on the leach resistance. The significant features of the test reported here on the Fingal glass are that (1) the glass was made on a semi-industrial scale using engineering appropriate to a production process and not under carefully controlled laboratory conditions; and (2)



the waste actually came from a nuclear reprocessing factory and was not synthetic. These satisfactory results give added confidence that vitrification processes now under development can be implemented on the full industrial scale with no loss in product performance, which is important for the successful management of highly active waste.

J. B. MORRIS  
K. A. BOULT  
J. T. DALTON  
M. H. DELVE  
R. GAYLER  
L. HERRING  
A. HOUGH  
J. A. C. MARPLES

*Atomic Energy Research Establishment,  
Harwell, Oxford, UK*

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## Interactions between nuclear waste and surrounding rock

DISSOLUTION and transport of radionuclides by groundwater are the main threats to the effective isolation of radioactive wastes by burial in deep geological formations. Calcine and glass are two solid forms which have been studied in this way for radioactive waste disposal. Long term release rates, based on extractions of radionuclides by simulated groundwater at 25 °C and atmospheric pressure, are projected to be very low from borosilicate glass, the most favourable candidate waste form. We present here evidence that contact with pressurised water at the 200–400 °C temperatures that could occur near the waste in the early years of storage rapidly alters the borosilicate glass and promotes interactions between waste and both shale and basalt repository wall rocks. The specific examples are the formation of a uranyl silicate (weeksite) directly from hydrothermal alteration of the glass and the reaction of caesium and sodium from calcine with aluminosilicates in basalt and shale to form the caesium sodium aluminosilicate, pollucite.

The materials sciences and experimental geosciences can provide risk assessments of a waste form, transportation system and isolation. These data help to evaluate the effectiveness with which the total system of a 'stable' solid waste form, engineered barriers and massive geological formations will assure that the longer lived radionuclides will decay sufficiently before reaching the biosphere in dangerous levels. The most probable way that radionuclides could be transported is by groundwater intrusion on the repository and there are two factors governing this transport: (1) the rate of release of the radionuclides from the nuclear waste solid and its engineered containment and (2) the rate of radionuclide migration through the various geological formations between the repository and the biosphere. Cohen<sup>1</sup> has reported that the radioactive decay combined with the rate of movement of deep groundwater and the adsorptive-exchange properties of rocks and soils should prevent radionuclides from reaching the biosphere at hazardous levels. Others<sup>2</sup>, however, question whether the geology alone can guarantee the effective isolation of radioactive wastes from the biosphere.

The form in which the wastes will be disposed has not been decided. It could be spent unprocessed fuel (SURF),

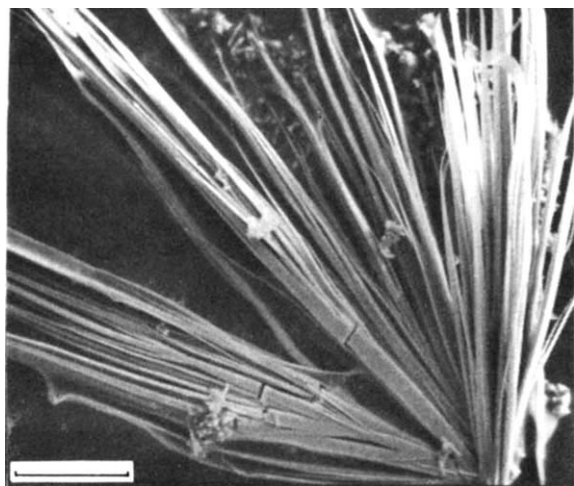
but if reprocessing is initiated then US regulations require that the resulting high level liquid wastes (HLLW) be solidified. Calcine, a granular solid or powder made by drying the HLLW at 500–800 °C, and borosilicate glass are currently the most likely solid form<sup>3</sup>, although crystalline-ceramic<sup>4</sup> and glass-ceramic<sup>5</sup> alternatives are also being developed. The most important property is the 'leachability', the susceptibility of a solid to removal of radionuclides by water. In most leachability tests the waste form is treated with water at 25 °C and one atmosphere<sup>6</sup>, and a very low rate of release of radionuclides into solution has been predicted for glass using such data<sup>7</sup>. This test, however, is irrelevant after the waste has been buried—the pressures, temperatures and chemical environment will be different, and the actual substance, whose long-term stability and non-leachability should be evaluated, might not be the original waste form but a reaction product between the waste form, its containment and the wall rock of the repository.

During the first few decades of geological isolation the heat of radioactive decay in the wastes could raise the immediate repository temperature to perhaps 300 °C in salt or 400 °C in other rocks<sup>8</sup>. Temperatures significantly above the normal 40–45 °C ambient for a 600–1,000 m deep repository would persist for up to several hundred years depending on where the waste containers are and the thermal conductivity of the host rock. If groundwater should contact the waste sealed in a repository during this thermal period of isolation, reactions would be expected to be very different than those observed in the 25 °C/1 atm IAEA leachability test. Poorly crystalline or noncrystalline solids such as glasses react readily with water under high temperature-high pressure (hydrothermal) conditions. (Desiccated gels (similar to calcines) and (powdered) glasses have been used as starting materials in hydrothermal synthesis and phase equilibria studies because they are so reactive<sup>9–11</sup>.)

Hydrothermal solutions would be expected to transport species to and from the waste solid and the host rock, a similar process to contact metamorphism. This would produce a zone around the waste container, a contact aureole, in which new 'minerals' which might not correspond to known mineral species could develop. The thickness of the aureole would be limited by the reduction of temperature away from the container. The phases in the aureole would depend primarily on the composition of the waste form and of the repository rock with perhaps some contribution from the container. Their effect on long-term fixation depends on their solubility in percolating groundwater. Largely crystalline waste solids would react far more slowly, but unless they were in thermodynamic equilibrium with the pressure-temperature-chemical environment of the waste-repository site, they too could eventually interact.

Once the brief (200–600 yr) thermal period ends, these interaction products, and the remnants of the waste form and its containment would constitute a 'new' waste form. This new waste form would act as source of radionuclides for any additional transport over the functional lifetime of the repository.

There are several hydrothermal experiments that confirm this waste-rock interactions. Spheroids (~4 mm) of one of the standard simulated waste borosilicate glasses (no. 76–68 provided by Battelle Northwest Laboratories; contains 13 wt % fission product oxides) were treated with a 10-fold excess by weight of water in a sealed gold capsule at ~300 °C and ~300 bar for one and two weeks. After one week the glass spheroid contained numerous fissures and several zones of coloration. After two weeks the specimen had broken into several fragments and seemed to be totally altered. The interior of the gold capsules was covered with crystals of the pyroxene mineral acmite, NaFeSi<sub>2</sub>O<sub>6</sub>; this alteration phase is reasonable because ~62 wt % of glass 76–68 consists of (Na<sub>2</sub>O + Fe<sub>2</sub>O<sub>3</sub> + SiO<sub>2</sub>). Millimetre-size radiating yellow needles were growing on

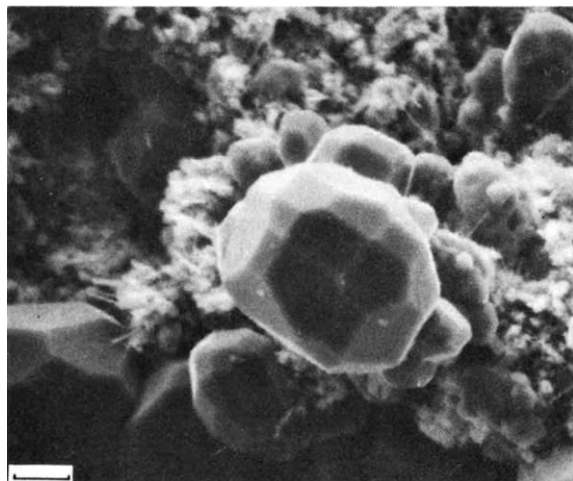


**Fig. 1** Crystals of a weeksite-structure phase,  $(\text{Na,Cs})_2(\text{UO}_2)_2(\text{Si}_2\text{O}_5)_3 \cdot 4\text{H}_2\text{O}$ , formed on the surface of a simulated radioactive waste glass during treatment with water at 300 °C, 300 bar for 7 d. Scale bar, 67  $\mu\text{m}$ .

the specimen and throughout the capsule. They have been identified from their elemental composition (by energy dispersive X-ray spectrometry), habit and X-ray powder data to be a uranyl mineral-like phase, Na–Cs–weeksite,  $(\text{Na,Cs})_2(\text{UO}_2)_2(\text{Si}_2\text{O}_5)_3 \cdot 4\text{H}_2\text{O}$  (Fig. 1). Atomic absorption spectrophotometry indicated that ~12 wt % of the Cs originally in the glass was taken into solution during the two week hydrothermal treatment. This is far more dissolved Cs than would be typically observed after two weeks of the IAEA test on a similar borosilicate glass specimen<sup>7</sup>.

In other experiments, simulated calcine (PW-7a)<sup>12</sup> was treated under hydrothermal conditions with specimens of a shale (Eleana Formation, Nevada) and of a basalt (USGS Columbia River Basalt Standard BCR-1) at 150–200 °C and ~300 bar for 7–30 d. Caesium and sodium in the calcine reacted readily with aluminosilicate minerals in the rocks to produce abundant white crystals. The crystal form observed by scanning electron microscopy (Fig. 2) and the elemental chemistry indicated that these crystals were Na–pollucite,  $(\text{Cs,Na})\text{AlSi}_2\text{O}_6 \cdot n\text{H}_2\text{O}$ .

**Fig. 2** Crystals of pollucite,  $(\text{Cs,Na})\text{AlSi}_2\text{O}_6 \cdot n\text{H}_2\text{O}$ , formed by interaction of Cs–Na-containing simulated radioactive waste calcine with Al and Si from a shale during hydrothermal treatment at 188 °C, 400 bars for 300 d. Scale bar, 3  $\mu\text{m}$ .



These preliminary experiments show that a waste–rock interaction under rather moderate temperature–pressure conditions will be rapid and extensive if the container is breached during the thermal period. The formation of Na–pollucite is also an example of a potentially desirable outcome of such interactions. The Cs in the waste calcine was probably in a water soluble solid form but Cs in  $(\text{Cs,Na})\text{AlSi}_2\text{O}_6$  is both effectively fixed<sup>13</sup> and in thermodynamic equilibrium with certain natural hydrothermal environments. Further work is required to determine whether the extraction from waste glasses of U as  $(\text{UO}_2)^{2+}$  would be observed under the reducing conditions of most groundwaters.

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G. J. MCCARTHY  
W. B. WHITE  
RUSTUM ROY  
B. E. SCHEETZ  
S. KOMARNENI  
D. K. SMITH  
D. M. ROY

Materials Research Laboratory  
and Department of Geosciences,  
The Pennsylvania State University,  
University Park, Pennsylvania 16802

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## Reinvestigation of the $\alpha$ -activity of Conway granite

ADAMS *et al.*<sup>1,2</sup> reported evidence for an unidentified 4.4 MeV  $\alpha$ -activity in certain core sections taken from Conway granite in New Hampshire. A similar  $\alpha$ -activity has also been reported by Cherdyntsev *et al.*<sup>3</sup> and by Brukl *et al.*<sup>4</sup> in different materials, but in neither case was it ever confirmed. We report here our reinvestigation of this phenomenon, and that we were unable to confirm the evidence of a 4.4 MeV  $\alpha$ -activity in the Conway granite.

Because it was thought that a previous failure<sup>5</sup> to confirm the existence of this activity in the Conway granite might have been due to subtle differences in sample preparation, considerable effort was made to obtain the same core material and to follow the same preparation techniques used by Adams *et al.*<sup>1,2</sup>. On the other hand, because no record was kept of the depth of the particular core section which yielded evidence for the 4.4 MeV  $\alpha$ -emitter, it is not known whether the Conway granite core sections used in



these experiments were from the same depth as the core section in question. Other pieces of granite from this same general area were also obtained from T. P. Kohman, and were given the same experimental treatment as the original cores obtained by Adams.

The experimental treatment, with minor variations from time to time, consisted of first crushing the 30.5 cm long, 2.5 cm diameter cores to about 1 mm size, followed by magnetic separation of the biotite in a standard Franz isodynamic magnetic separator. The biotite flakes obtained from this magnetic separation were then crushed using a mortar and pestle. To extract the small radioactive inclusions from this crushed biotite, a heavy liquid separation was carried out using methylene iodide ( $\rho = 3.4 \text{ g cm}^{-3}$ ). The crushed biotite was left in the methylene iodide for several hours to allow ample time for the small (10–200  $\mu\text{m}$ ) radioactive mineral inclusions ( $\rho > 3.5 \text{ g cm}^{-3}$ ) to separate from the less dense biotite ( $\rho \approx 3 \text{ g cm}^{-3}$ ). The methylene iodide residue was subsequently removed from the inclusions using an acetone rinse.

After drying, the inclusions were sprinkled on to the surface of 1×3 inch Eastman Kodak NTA emulsion track plates. These NTA plates had an emulsion thickness of 25  $\mu\text{m}$  and were sensitive to  $\alpha$  particles without recording  $\beta$  rays. The plates with inclusions were then placed in a light tight box under refrigeration for periods ranging from three weeks to three months. Subsequent development and microscopic scanning of these plates revealed the  $\alpha$ -activities of the various individual inclusions on the plate. At this point microscopic techniques were utilised to pick out only the inclusions which exhibited the greatest cluster of  $\alpha$  tracks. These highly radioactive inclusions, which varied in size from about 10 to 200  $\mu\text{m}$ , were then placed either singly or in groups of up to 25 on to Pt or stainless steel disks. The initial experiments involved dissolution of these inclusions on the Pt disks with drops of concentrated HF and HNO<sub>3</sub>, the Pt disk itself serving as the source for the  $\alpha$ -spectrometer. It was soon found that better energy resolution could be obtained by subsequent crushing of the dissolved residue, and this technique was followed until it was found that the acid dissolution could be dispensed with entirely. From then on, the higher activity inclusions were mounted on stainless steel disks, crushed, and then counted for periods ranging from about one day to a week.

The measurements were made on an  $\alpha$ -spectrometer with a 2 cm diameter, gold-covered surface barrier Si-detector of 300  $\mu\text{m}$  depth mounted in a vacuum chamber. Sample disks were placed in the bottom of a polyethylene cap which fitted over the detector so that the sample was approximately 1 mm from the face of the Si-diode. A preamp was mounted directly on the base of the detector (bias  $\sim 100 \text{ V}$ ) and its output was fed to an amplifier. The pulse-height spectrum was measured with a multichannel analyser. Although the system exhibited a resolution of 30–35 keV (FWHM) for a very thin source, samples prepared by the above technique exhibited a typical resolution of 50–60 keV. Measurements generally spanned the range of  $\sim 2$  to 10 MeV over 2,048 channels. Energy drift and background were negligible.

About 50 different  $\alpha$ -spectra were obtained on the various cores and samples from the Conway granite. The  $\alpha$ -spectra from the multichannel analyser were recorded on punched tape, which then, by means of a computer program, generated an accurate plot of counts per channel against channel number. Energy calibration was accomplished by means of a pulser and standard  $\alpha$ -sources.

The  $\alpha$ -spectra generally showed evidence of both the  $^{238}\text{U}$  and  $^{232}\text{Th}$   $\alpha$ -decay chains, and in some cases the abundance ratios of the two elements and their respective daughters were noted to vary from inclusion to inclusion. This was not surprising, for the inclusion selection process was not specific for a particular mineral, hence different

U/Th ratios were to be expected as dissimilar mineral inclusions were analysed. In some cases disequilibrium in the U–Th chains was observed in the  $\alpha$ -spectra but no attempt was made to determine whether this condition arose from slight variations in the sample preparation procedure or from an inherent disequilibrium within the sample before crushing. The reason for this disequilibrium condition was not pursued because the main point of the experiments was to determine whether any 4.4 MeV  $\alpha$ -activity could be detected.

With the possible exception of one sample, which exhibited poor statistics because of a relatively weak source, the experiments did not reveal any evidence for the 4.4 MeV  $\alpha$ -emitter reported by Adams *et al.*<sup>1</sup>. A redetermination of that particular sample subsequently gave no evidence whatsoever for the 4.4 MeV activity. Thus we were unable to find any confirmatory data for the existence of a 4.4 MeV  $\alpha$ -activity in the Conway granite.

This research was supported by Union Carbide Corporation under contract with the Division of Basic Energy Sciences of the Department of Energy. Separation of the biotite from the crushed granite was carried out using the magnetic separator located in the Geology Department of the University of Tennessee, Knoxville. Mr Mirza Beg and later Dr Otto Kopp supervised this phase of the experiment.

R. V. GENTRY  
J. H. HALPERIN  
B. H. KETELLE  
G. D. O'KELLEY  
R. W. STOUGHTON

Chemistry Division,  
Oak Ridge National Laboratory,  
Oak Ridge, Tennessee 37830

J. A. S. ADAMS

Geology Department,  
Rice University,  
Houston, Texas 77001

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## Evidence of large Cainozoic crustal shortening of Asia

PALAEOMAGNETIC studies of late Cretaceous–early Tertiary red beds in southern Tibet indicate that they were magnetised at about 8°N and, therefore, that they subsequently moved north about 22° (ref. 1). Xiangyuan *et al.*<sup>1</sup> collected 12 samples across the thickness of red beds which overlies an early late-Cretaceous stratum (Tsu mulong group) in Linzhou. Fossils of late Cretaceous marine fauna were found within the red bed itself, and Foraminifera (*Lockhatia* sp.) of early Tertiary age were found near an outcrop of the volcanic assembly that unconformably overlies the red beds. Three of their samples were tested for stability using alternating field demagnetisation with an applied field of 50–300 oersted. No obvious secondary magnetisation was found. These red beds are north of the Indus–Tsangpo suture<sup>2</sup>. Xiangyuan *et al.*<sup>1</sup> compared the virtual geomagnetic pole from Tibet with those from rocks of the same age in

Table 1 Palaeomagnetic poles from Eurasian samples

| Site location (and age)                      | Palaeomagnetic pole ( $^{\circ}$ N, $^{\circ}$ E) |       | Palaeolatitude* | Difference† (km) |
|----------------------------------------------|---------------------------------------------------|-------|-----------------|------------------|
| Tibet 30°N 91.3°E (early Tert. – late Cret.) | 67.5                                              | 283.1 | 8°              |                  |
| Spitzbergen 79°N 15°E (late Cret.)           | 75.0                                              | 235.0 | 18°             | 10° (1,100)      |
| Czechoslovakia 50.9°N 13.4°E (Cret.)         | 78.7                                              | 186.9 | 28°             | 20° (2,250)      |
| Siberia 56.5°N 89.5°E (Cret.)                | 74.0                                              | 135.0 | 41°             | 33° (3,600)      |
| Siberia 71°N 111°E (Cret.)                   | 66.0                                              | 170.0 | 32°             | 24° (2,600)      |
| Western Europe (early Tert. average)         | 75.0                                              | 151.0 | 36.5°           | 28.5° (3,200)    |
| Western Europe (Cret. average)               | 86.0                                              | 00.0  | 30°             | 22° (2,400)      |
| Russian Platform (early Tert. average)       | 68.0                                              | 192.0 | 24°             | 16° (1,800)      |
| Eurasia (70 Myr)                             | 75.0                                              | 170.0 | 32°             | 24° (2,600)      |
| Eurasia (60 Myr)                             | 75.0                                              | 175.0 | 30.5°           | 22.5° (2,500)    |

\*Palaeolatitudes equal 90° minus distances of palaeomagnetic poles from 30°N 91.3°E.

†The differences in these palaeolatitudes from that measured for southern Tibet<sup>1</sup> give the estimated northwards convergence of southern Tibet with respect to Eurasia.

India to show that this portion of Tibet was indeed not part of India in the late Cretaceous–early Tertiary. We show here that these new palaeomagnetic data also require about 2,500–3,500 km of north–south shortening in Asia since its collision with India.

The amount of relative latitudinal motion between southern Tibet and Eurasia is the difference between southern Tibet's palaeolatitude and the latitude of southern Tibet's present position measured relative to Eurasia's late Cretaceous–early Tertiary pole. Note that a rotation of samples in southern Tibet does not affect this estimate. Table 1 lists various palaeomagnetic poles (Fig. 1) from Eurasia samples and the corresponding palaeolatitudes for the present position of southern Tibet. Individual poles are the most reliable ones listed by Irving *et al.*<sup>3</sup>, and the various averages are from McElhinny<sup>4</sup> and Irving<sup>5</sup>. Among them the most reliable are probably Irving's<sup>5</sup> apparent polar wander curves and the early Tertiary average of Europe, which is based on several consistent studies of the early Tertiary igneous rocks of the British Isles and surrounding regions. These imply relative convergence of southern Tibet and Eurasia of about 2,500 and 3,200 km, respectively. Note that Irving's polar wander curve since the late Cretaceous is nearly parallel to the 180° meridian, and the Tibetan

samples are from near the 90°E meridian. Therefore, if Irving's curve is reliable, the uncertainty in age of the Tibetan samples does not affect the calculated convergence between them and Eurasia.

Although Mesozoic orogenic activity is implied by unconformities in middle Cretaceous sedimentation<sup>6</sup>, by granites of Mesozoic age<sup>7</sup>, and by ophiolites north of the Indus–Tsangpo suture<sup>8</sup>, the widespread late Cretaceous limestones and sandstones covering most of Tibet suggest that it was a stable platform when these sediments were deposited<sup>9,10</sup>. Our ignorance of Tibetan geology does not allow a categorical denial of the existence of oceanic lithosphere between Tibet and Siberia since the late Cretaceous, but we have no evidence for it. Thus we presume that all northwards motion of southern Tibet with respect to Eurasia (Fig. 2) would involve deformation and displacement of only continental material. In reconstructions of India and Eurasia at the time of their collision 40–50 Myr ago, India lay 2,000–3,000 km south of its present position relative to Eurasia<sup>10</sup>, still 3,000 km further south, 65 Myr ago (Fig. 2). Therefore, the palaeomagnetic data and the reconstruction agree with one another, without the need for postulating a large amount of underthrusting of India beneath Eurasia.

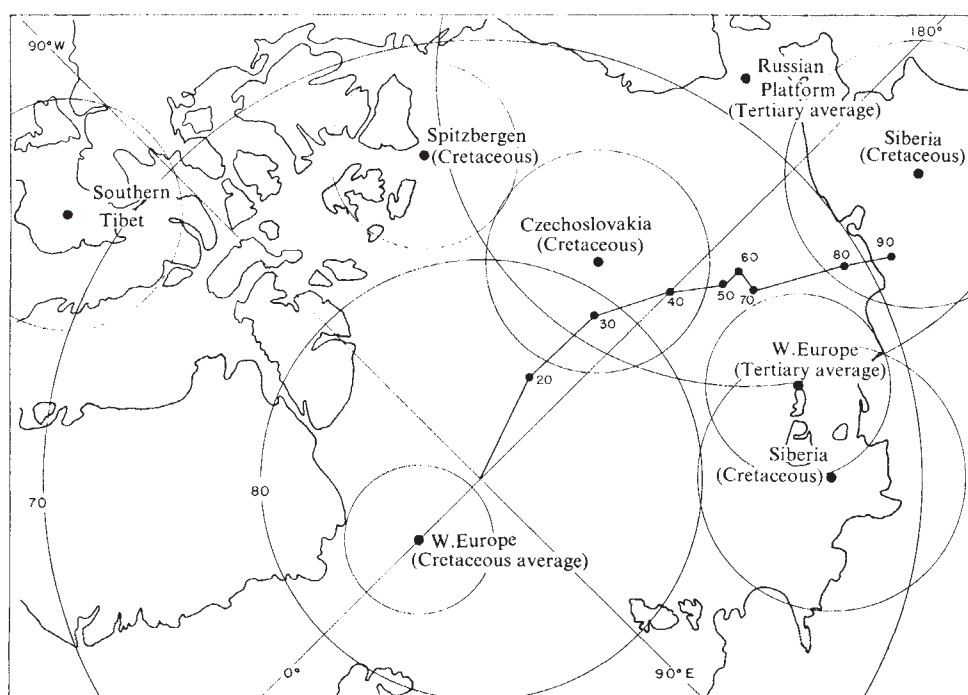
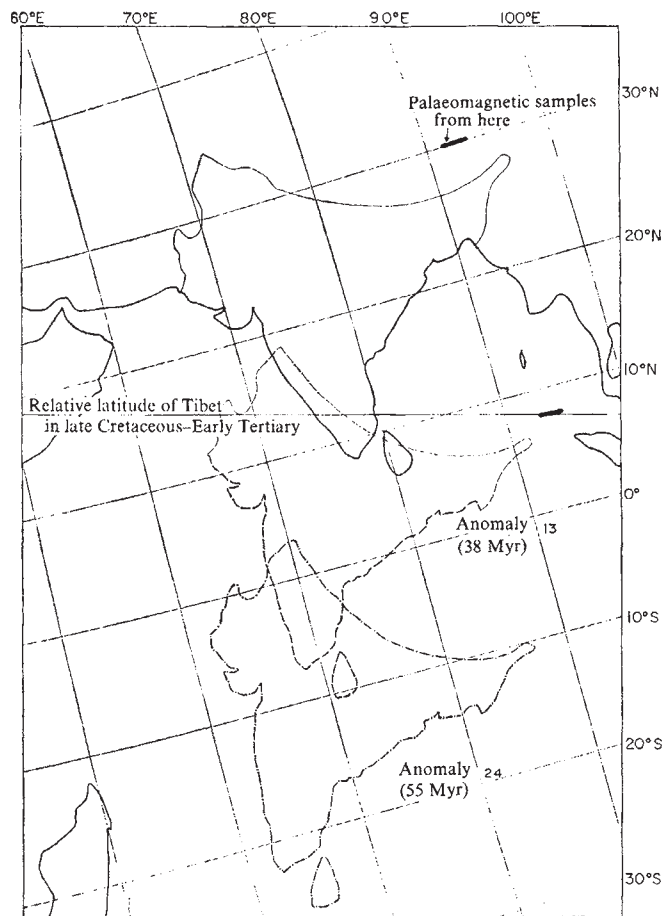


Fig. 1 Palaeomagnetic poles and 95% confidence regions for Tibet<sup>1</sup> and for selected Eurasian studies<sup>3</sup> and averages<sup>4,5</sup>. Apparent polar wander wave for Eurasia<sup>5</sup> is given with numbers in Myr next to each point. As samples in southern Tibet lie on the 90° meridian, palaeolatitudes may be estimated directly from distances to the 0–180° meridian.





**Fig. 2** Oblique Mercator projection of southern Asia with pole at 75°N, 172.5°E, Irving's<sup>5</sup> pole for Eurasia at 65 Myr ago. The possible positions of Tibet relative to Eurasia assuming that the rocks were magnetised 65 Myr ago are shown, and the dark bar at the present site of Tibetan samples is rotated to its palaeolatitude at 65 Myr ago to show rotation of site about an axis through it. India is reconstructed<sup>11</sup> to its positions at the times of anomaly 13 (38 Myr) and 24 (55 Myr). Note that the northern margin of India is arbitrarily taken to be the Himalayan front because an unknown amount of shortening has taken place north of it in the Himalayas. The northern margin of India probably lay about 600 km north of the present front. Therefore, the collision would have occurred before 38 Myr ago.

Dan McKenzie first called our attention to the new palaeomagnetic data<sup>1</sup>, and the research was supported by the US NSF.

PETER MOLNAR  
WANG-PING CHEN

Department of Earth and Planetary Sciences,  
Massachusetts Institute of Technology,  
Cambridge, Massachusetts 02139

Received 16 January; accepted 22 March 1978.

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## Palaeogeotherms: implications of disequilibrium in garnet lherzolite xenoliths

MEASURES of the Earth's thermal structure and evolution are important for understanding the driving forces of many geological processes. Estimates of the present variation of temperature with depth in the Earth can be made from surface heat-flow values together with assumptions concerning the composition, thermal conductivity and heat production of the crust and mantle<sup>1</sup>. More direct estimates have been made by calculating equilibrium temperatures and pressures of material brought to the Earth's surface as nodules in kimberlite pipes<sup>2,3</sup> and volcanoes<sup>4</sup>. Results from the latter studies have been used to describe the equilibrium distribution of temperature and pressure within the Earth<sup>3</sup> at times from the Precambrian<sup>5–8</sup> to the late Mesozoic<sup>9</sup>. Although there has been much discussion of the exact shape<sup>3,10–12</sup> and significance<sup>13,14</sup> of the calculated geothermal gradients, it has generally been assumed that the calculated values of temperature and pressure are estimates of the true equilibrium conditions of the assemblage. Here the basic assumption of chemical equilibrium is examined critically and it is shown that the calculated geotherms may be at least partly spurious. The distribution in calculated temperatures and pressures can be explained by the inherent temperature dependence of the geobarometer and the failure of the assemblages to equilibrate with respect to all components.

Equilibrium temperatures and pressures have been calculated for suites of garnet lherzolite nodules, from the Bultfontein (P. J. Lawless, unpublished) and Premier (R. V. Danchin, unpublished) mines in South Africa (Fig. 1). Nodules from other localities in Southern Africa lie on similar geotherms<sup>10</sup>. Temperatures were obtained from the compositions of co-existing pyroxenes using the best-fit two-pyroxene geothermometer of Wells<sup>15</sup>. Effects of pressure on the diopside–enstatite miscibility gap<sup>12,16,17</sup> were neglected as such effects do not alter the conclusions of this work. Pressures were estimated using the orthopyroxene–garnet geobarometer of Wood<sup>18</sup>:

$$(P-1) \Delta V_{rn} = 7,010 - 3.89T - RT \ln K$$

$$\text{where } K = (X_{Mg}^{gt})^3 (X_{Al}^{gt})^2 / (X_{Mg}^{M2})_{opx}^2 (X_{Mg}^{M1})_{opx} (X_{Al}^{M1})_{opx}$$

It should be emphasised that the calculated pressure is strongly dependent on the temperature obtained from the two-pyroxene geothermometer.

If the data shown in Fig. 1 describe the true equilibrium conditions for each nodule, the calculated distribution of temperatures and pressures reflects the contemporary geothermal gradient sampled at different depths during the rapid ascent of the kimberlite. It is, however, important to demonstrate the existence of chemical equilibrium in the nodules. Figure 1 also shows the inherent temperature dependence of the geobarometer (see equation (1)) for different values of  $K$ . It can be seen that the calculated temperatures and pressures lie on lines of constant  $K$ . When  $Al^{3+}$  exchange for a given garnet–orthopyroxene pair ceases with falling temperature a particular value of  $K$  will be 'frozen in' to the assemblage. If, however,  $Ca^{2+}$  and  $Mg^{2+}$  continue to diffuse between pyroxenes below this temperature, or at least diffuse more rapidly, the two-pyroxene geothermometer will continue to monitor falling temperatures although the geobarometer has been locked in with a fixed value of  $K$ . Further changes in temperature will, therefore, cause the data points to move along lines of constant  $K$  thus generating a spurious apparent geotherm.

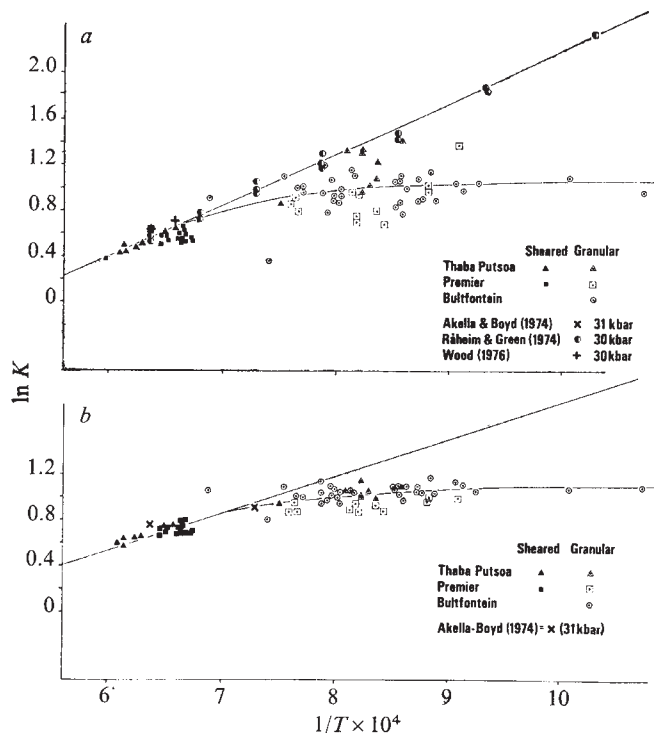
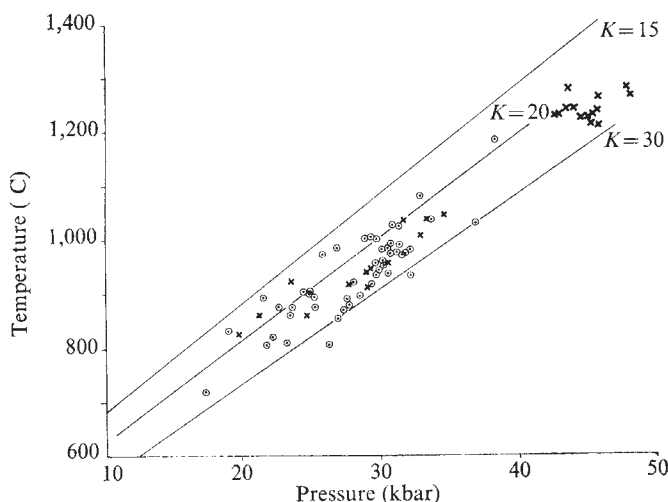
To test the possibility that diffusion between pyroxenes and garnets may be less rapid than between the pyroxenes themselves, equilibrium constants for  $Mg$ – $Fe^{2+}$  exchange between orthopyroxene and garnet and between clinopyroxene and garnet were calculated and are plotted against the temperature obtained from the two-pyroxene geothermometer in Fig. 2.

These data show the interesting result that  $\text{Mg-Fe}^{2+}$  exchange between pyroxenes and garnet and  $\text{Ca-Mg}$  exchange between the two pyroxenes are coherent for the high-temperature group of sheared nodules. However, while temperature changes below  $1,100^\circ\text{C}$  continue to be monitored by changing pyroxene positions, re-equilibration of  $\text{Mg/Fe}^{2+}$  distribution between pyroxenes and garnet no longer takes place below about  $1,100^\circ\text{C}$  for the coarser, granular nodules. This refractory behaviour of garnet leading to quite constant values of  $\text{Mg-Fe}^{2+}$  distribution coefficients between pyroxene and garnet despite changing temperatures has been observed elsewhere<sup>19</sup>. Although  $\text{Mg/Fe}^{2+}$  ratios of peridotitic garnets alone may reflect changes in Ca content<sup>19</sup> the distribution coefficient used here refers to an exchange reaction between two phases, both of which will respond to changes in Ca content. In the absence of large differences between non-ideal  $\text{Ca-Mg}$  and  $\text{Ca-Fe}$  interactions, garnet-pyroxene  $\text{Mg-Fe}^{2+}$  distribution should be insensitive to changing bulk composition<sup>20</sup> and this has been shown experimentally for a wide range of compositions<sup>21</sup>.

Diffusion of  $\text{Al}^{3+}$  between orthopyroxene and garnet will also be kinetically limited. However, because of the higher charge<sup>22</sup> and different diffusion mechanism involving a gross structural transformation in the growth of garnet from aluminous pyroxenes, the effective blocking temperature below which Al diffusion ceases in the nodules should be higher than observed for the diffusion of Mg and  $\text{Fe}^{2+}$ . The immobility of  $\text{Al}^{3+}$  during metamorphic reactions has been noted elsewhere by Carmichael<sup>23</sup>. Such kinetic differences would cause the two-pyroxene geothermometer and the garnet orthopyroxene geobarometer to be out of phase with one another thus generating a spurious apparent geotherm.

The factors affecting the observed blocking temperature will clearly include grain size, contemporaneous shear stress, and rate of cooling, and these are being investigated by detailed petrographic study. Whereas much debate has centred upon whether some palaeogeothermal gradients are perturbed or not, it is important to establish to what extent temperature-pressure distributions calculated from garnet lherzolite nodules represent true equilibrium conditions in the mantle and how far these may be modified by the effects of different blocking temperatures for the geothermometer and geobarometer as discussed above.

**Fig. 1** Apparent geotherm described by nodules from the Bultfontein and Premier Mines, South Africa. The calculated temperatures and pressures lie along the temperature dependence of the garnet-orthopyroxene geobarometer. Changes in temperature indicated by the two pyroxene compositions cause the data points to move along lines of constant  $K$ . The Precambrian Premier data and the late Mesozoic-Bultfontein data lie on the same apparent geotherm. X, Premier; O, Bultfontein.



**Fig. 2** Comparison of  $\text{Mg-Fe}^{2+}$  distribution in clinopyroxene-garnet and orthopyroxene-garnet pairs with experimental data. The high temperature sheared nodules are equilibrated and in phase with the two-pyroxene geothermometer. However, the coarser granular nodules have not equilibrated with respect to pyroxene-garnet compositions although the two-pyroxenes themselves continue to respond to falling temperatures. Cpx, clinopyroxene; opx, orthopyroxene; gt, garnet. *a*,  $K = (\text{Mg/Fe})_{\text{cpx}}/(\text{Mg/Fe})_{\text{gt}}$ ; *b*,  $K = (\text{Mg/Fe})_{\text{opx}}/(\text{Mg/Fe})_{\text{gt}}$ .

Until these factors are understood in detail, all geotherms calculated from the equilibrium temperatures and pressures of pyroxene-garnet assemblages should be used with caution.

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DONALD G. FRASER

Department of Geology and Mineralogy,  
University of Oxford,  
Parks Road,  
Oxford, UK

P. J. LAWLESS

Anglo-American Research Laboratories,  
P.O. Box 106,  
Crown Mines, 2025,  
South Africa

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## A failed Gondwana spreading axis in southern Africa

AN outstanding geological feature revealed by the recently published Reconnaissance Aeromagnetic Survey of Botswana<sup>1</sup> is a well defined swarm of closely spaced dykes crossing the northern part of the country on a strike of approximately N 110°E (Fig. 1). We present here an interpretation of this newly discovered dyke swarm in terms of a failed Gondwana spreading axis.

The swarm crosses the width of Botswana without interruption or diminution, and individual dykes may be traced with some confidence over distances of several hundred kilometres. Both Archaean Basement and Damaran meta-sediments are cut without regard to the grain of the country rock. The swarm is closely confined between boundaries which converge to the west, the swarm being 110 km wide at longitude 27°E and 55 km wide at the border with Namibia (longitude 21°E). Both the Okavango Delta and the Makgadikgadi Salt Pans lie astride the swarm. More widely spaced airborne survey data east of 27°E indicate that the swarm continues eastwards as far as the eastern boundary of Botswana with Rhodesia, a distance of over 800 km.

The abundance of dykes striking N 110°E in the northern part of Botswana has been recognised for some time from isolated aeromagnetic surveys<sup>2</sup>. Within Botswana, but outside the area of Kalahari Sand cover (that is, east of the eastern Makgadikgadi shoreline), geological mapping and photogeological interpretation have identified dykes with this strike direction cutting the Stormberg Basalts, the youngest member of the Karroo sequence, at some localities<sup>3–6</sup>. Here there is good agreement between field observation and the aeromagnetic data, but it is clear that the latter is the more sensitive technique for mapping dykes. Consequently, the national aeromagnetic coverage reveals the remarkable geometrical arrangement of the dyke swarm as a whole.

When the dyke swarm is projected 300 km to the east, beyond the survey coverage, it converges with two well-known lines of post-Karoo tectonism, namely the Lebombo and Sabi Monoclines (Fig. 1). The three axes intersect at angles of almost precisely 120°. Both the Lebombo and Sabi Monoclines are thought to be structurally related to the disruption of Gondwanaland, if not themselves actual continental margins<sup>7</sup>. Burke and Dewey<sup>8</sup> quote this locality as one of several examples around the margin of Africa where Gondwanaland disruption initiated as a rift–rift–rift triple junction, one arm of which subsequently ceased to develop before the spreading stage, the other two forming the boundary between divergent plates. The newly discovered dyke swarm seems to be a more significant manifestation of this failed third arm than their loosely defined 'Limpopo Rift'.

The geology of the Limpopo valley has been described in some detail and post-Karoo dykes striking predominantly N 100°E have been recognised in the Tuli Syncline<sup>9</sup>, and striking N 70°E, N 110°E and N–S in the Nuanetsi post-Karoo Igneous Province<sup>10</sup>. A more complex nature of dyke distribution in the closer vicinity of the triple junction is also indicated by aeromagnetic data for the most northerly

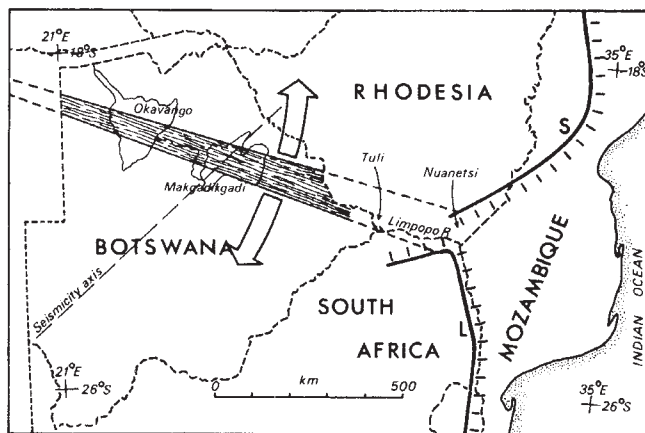


Fig. 1 The post-Karoo dyke swarm in Botswana, showing its relationship to the Lebombo Monocline (L) and the Sabi Monocline (S). Direction of relative plate rotation leading to dyke injection indicated by arrows.

areas of South Africa<sup>11</sup>. Unfortunately, no aeromagnetic data are available for southern Rhodesia to delineate more closely the disposition of the main swarm as it enters the area of the ancient Limpopo Mobile Belt (trending N 70°E) and approaches Nuanetsi.

The narrowing of the dyke swarm to the west within Botswana indicates that its sides would finally converge somewhere north of Etosha Pan in Namibia. This suggests that the slight degree of opening experienced by this vestigial spreading axis propagated westwards from the triple junction, opening in a hinge-like manner, as indicated in Fig. 1. Sympathetic propagation resulting in release of stress between adjacent rigid plates seems to be more likely than a driven opening resulting from mantle processes beneath the dyke swarm, but actual crustal dilation between the plates must have been minimal, as there is continuous basement exposure across the swarm in eastern Botswana and the Limpopo valley.

From the Karroo geology of Botswana<sup>4</sup>, lower members of the Karroo sedimentary sequence, as well as the capping Stormberg Basalt, seem to be noticeably thin or absent along the axis of the dyke swarm, indicating either uplift and limited sedimentation during Karroo deposition, or subsequent uplift and erosion of the Karroo. By contrast, the dyke swarm passes through what are now the topographically lowest parts of the Kalahari inland drainage basin (namely the Makgadikgadi Salt Pans and the Okavango Delta), and the Limpopo valley. At the former two localities the dyke swarm intersects, respectively, the north-easterly trending Kalahari Seismicity Axis<sup>12</sup> and the more intense seismicity of the Okavango Delta<sup>13</sup>. From the north-easterly strike direction of the major faults associated with these features, and fault plane solutions for minor earthquakes in the Okavango<sup>13</sup>, these features seem to be primarily related to a pattern of more recent (Tertiary to Recent) rifting extending from the north-east<sup>14</sup>, and only secondarily, if at all, to the obsolescent dyke swarm axis, which was presumably of short-lived tectonic importance.

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C. V. REEVES

*Terra Surveys,  
2060 Walkley Road,  
Ottawa, Ontario, Canada*

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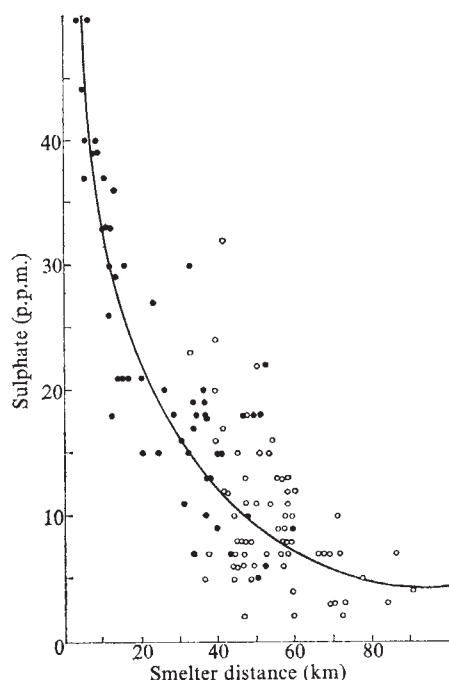
## Isotopic variation as an index of sulphur pollution in lakes around Sudbury, Ontario

SULPHUR isotopic variations can in principle be used in fingerprinting the sources of sulphur and for tracing the pathways of sulphur migration in the environment (for examples, see refs 1–4). However, few attempts have been made to apply this technique in the study of sulphur pollution of natural waters. We present here results of a study of the distribution and isotopic composition of sulphur in lakes at various distances from the smelter stacks near Sudbury, Ontario. An objective of the study is to ascertain the point source contribution to variations in the isotopic composition of sulphur in these lakes.

The INCO nickel smelter stack at Copper Cliff, near Sudbury, is about 380 m tall and emits approximately 35,000 tons of  $\text{SO}_2$  daily<sup>5</sup>, or roughly 20% of all the  $\text{SO}_2$  emitted annually in Canada<sup>6</sup>. The plume from the stack usually rises to about 1,000 m after emission and occasionally travels for hundreds of kilometres downwind in a coherent form<sup>5</sup>. The Sudbury area has encountered environmental problems attributable to the smelter stack emissions. For example, the acidification of lakes around Sudbury has been documented in several studies<sup>7–10</sup>.

The study area chosen encompassed the quadrant lying between the south and west of Copper Cliff and extending for a distance of up to 100 km. This area spans two geological areas: in the south, orthoquartzite ridges forming the La Cloche

Fig. 1 Variation of sulphate concentrations with distance of the lakes from the smelter stack at Copper Hill near Sudbury, Ontario. The line of least squares fit is indicated.



Mountains, and to the north, the flatter terrain dominated by glacial tills, conglomerate, arkose, orthoquartzite, and so on.

The initial survey involved sampling some 67 lakes in the La Cloche Mountains and 54 lakes to the north of these. The pH was measured in the field using a Sargent-Welch Model PBL meter with a Corning low-ionic strength probe and reference electrode. Sulphate was determined in the field using the Hach DRL-300 kit.

On the basis of the results of this survey, a sub-set of 41 lakes was chosen for more detailed investigation in 1975. The pH was again measured promptly in the field as above. The sulphate contents of the latter samples were determined gravimetrically as barium sulphate. The barium sulphate was thermally decomposed to liberate  $\text{SO}_2$  using the method of

Table 1 Chemical parameters for lakes near Sudbury, Ontario

| Lake             | Smelter distance (km) | pH   | Sulphate level (p.p.m.) | $\delta^{34}\text{S}$ (‰) |
|------------------|-----------------------|------|-------------------------|---------------------------|
| Robinson L.      | 3.2                   | 6.09 | 30.5                    | +8.46                     |
| Hannah L.        | 3.9                   | 3.40 | 53.0                    | —                         |
| St. Charles      | 4.5                   | 4.53 | 35.4                    | +3.48                     |
| Nepohwin         | 5.2                   | 6.15 | 36.1                    | +7.32                     |
| "e"              | 5.2                   | 3.20 | 23.5                    | +5.19                     |
| Middle           | 5.2                   | 5.78 | 39.5                    | +2.66                     |
| Silver           | 6.5                   | 3.20 | 28.5                    | +5.19                     |
| Ramsay           | 7.7                   | 6.61 | 34.7                    | +7.34                     |
| Richard          | 8.4                   | 5.06 | 25.7                    | +8.43                     |
| McFarlane        | 9.7                   | 5.32 | 27.4                    | +6.76                     |
| Lohi             | 10.3                  | 4.20 | —                       | —                         |
| Long             | 11.0                  | 6.45 | 22.3                    | +5.31                     |
| Raft             | 11.6                  | 4.15 | 20.9                    | +2.84                     |
| Clearwater       | 12.9                  | 3.50 | 24.4                    | +4.56                     |
| Tilton           | 13.5                  | 4.20 | 19.3                    | +4.50                     |
| Makada           | 14.8                  | 6.75 | 21.8                    | +4.23                     |
| McCharles        | 20.0                  | 8.95 | 87.8                    | +3.57                     |
| Wavy             | 20.0                  | 3.30 | 15.8                    | +5.23                     |
| Little Panache   | 32.3                  | 8.71 | 13.7                    | +6.08                     |
| Panache          | 32.9                  | 6.70 | 19.9                    | +4.70                     |
| Broker           | 37.4                  | 5.20 | 7.8                     | +4.75                     |
| Tyson            | 39.4                  | 5.70 | 12.9                    | +4.84                     |
| Log Boom         | 41.9                  | 5.19 | 12.9                    | +4.80                     |
| Johnnie          | 45.2                  | 4.15 | 11.0                    | +4.30                     |
| Ruth-Roy         | 45.2                  | 4.50 | 12.8                    | +4.05                     |
| Norway           | 47.1                  | 4.20 | 12.3                    | +4.00                     |
| Perdix           | 47.7                  | 4.41 | 14.6                    | +4.99                     |
| Carlyle          | 49.0                  | 4.85 | 11.9                    | +4.99                     |
| Kakakise         | 50.3                  | 5.75 | 13.6                    | +4.48                     |
| Lang             | 52.3                  | 6.75 | 20.3                    | +4.67                     |
| Acid (Lum II)    | 57.4                  | 4.39 | 9.5                     | +4.48                     |
| Lumsden I        | 58.1                  | 4.39 | 8.9                     | +4.55                     |
| Lumsden III      | 58.1                  | 4.60 | 8.9                     | +5.71                     |
| Apsey            | 61.3                  | 7.01 | 10.8                    | +5.43                     |
| Froody           | 62.6                  | 6.70 | 17.6                    | +4.82                     |
| Grab             | 67.0                  | 6.25 | 8.1                     | +5.26                     |
| Evangeline       | 73.6                  | 6.42 | 9.7                     | +5.36                     |
| Maple            | 75.5                  | 6.40 | 12.1                    | +4.46                     |
| Cutler           | 76.7                  | 6.79 | 12.1                    | +4.22                     |
| La Cloche        | 83.9                  | 6.68 | 11.2                    | +5.03                     |
| Little La Cloche | 85.8                  | 6.80 | 13.2                    | +4.34                     |
| Owl              | 90.3                  | 4.75 | 8.1                     | +4.73                     |

Holt and Engelkemeir<sup>11</sup>. The  $^{32}\text{S}/^{34}\text{S}$  ratios in the  $\text{SO}_2$  samples were determined on a dual-collector, isotope ratio mass spectrometer (VG Micromass, Model 602C). The isotope ratio results are reported in the usual  $\delta^{34}\text{S}$  notation:

$$\delta^{34}\text{S} (\text{‰}) = (R_{\text{sample}}/R_{\text{std}} - 1) \times 1,000$$

where  $R_{\text{sample}}$  and  $R_{\text{std}}$  refer to the  $^{34}\text{S}/^{32}\text{S}$  ratios of the sample and the standard Canyon Diablo troilite respectively. The overall precision of the  $\delta^{34}\text{S}$  data reported is  $\pm 0.2\text{‰}$  based on replicate analyses of several lake water samples.

In the preliminary survey, the sulphate concentration in the 120 lakes was highly correlated inversely with the distance from the smelter at Copper Cliff ( $P = 0.86$ ). Sulphate levels were high, up to a distance of some 60 km (see Fig. 1). This is in contrast to the findings of Gorham and Gordon<sup>7</sup> who noted



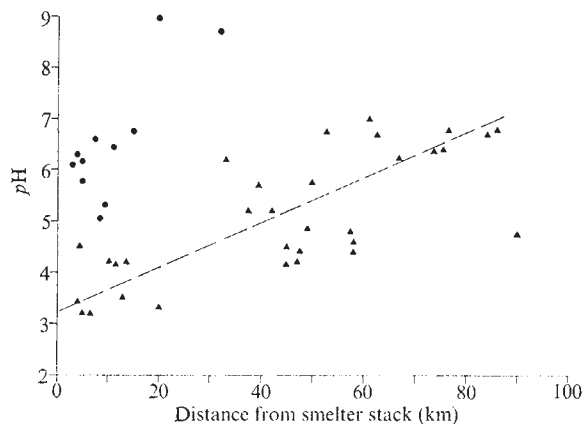


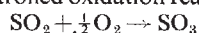
Fig. 2 Changes in pH with distance of lakes from the smelter stack.  $\Delta$ , Poorly buffered lakes;  $\bullet$ , well buffered lakes. The broken line which fits the poorly buffered lakes was drawn by eye.

that 'Outside about 15 miles distance the influence of smelter pollution upon sulphate concentration in surface waters is negligible.' The calculated curve of best fit (see Fig. 1) indicates a concentration of 10 p.p.m. of  $\text{SO}_4$  at 50 km, and that the concentration continues to decline even at a distance of 90 km from the smelter. The La Cloche Mountains end some 90 km west of Copper Cliff, and sampling of lakes in this formation could not be continued further.

The group of 41 lakes in which the sulphate concentrations fall close to the regression line was sampled again during 1975. Table 1 summarises the chemical and isotopic data for these lakes. The sulphate versus distance graph can be described by the expression,  $y = 55x^{-0.38}$ , with  $r = 0.81$ . On the basis of the observed pH, lakes in the latter group can be subdivided into (1) well buffered lakes unresponsive to the input of acid precipitation associated with the smelter emissions; (2) poorly buffered lakes more easily influenced by the smelter emissions. As indicated in Fig. 2, there is a significant correlation between the pH of the poorly buffered lakes and the distance from the smelter track. The acidification of freshwaters is a difficult problem which will not be considered here (see ref. 12 for a review).

The  $\delta^{34}\text{S}$  values of the lakes at varying distances from the stack are shown in Fig. 3. The  $\delta^{34}\text{S}$  data for the well buffered lakes show considerable scatter, suggesting (1) derivation of the sulphur from several sources; (2) complex interplay between the point source influence and the transformations of the sulphur either in the watershed or within the lakes, or (3) a combination of these factors. With regards to the wide dispersion in the  $\delta^{34}\text{S}$  data, it is notable that accelerated weathering associated with high acid loading may increase the amount of sulphur in these lakes contributed from the bedrock and the overburden. Another important feature of Fig. 3 is that the  $\delta^{34}\text{S}$  data for the poorly buffered lakes are remarkably uniform, lying mostly between 4.5 and 5.5‰. This uniformity can only suggest (see below) that the isotopic composition of these lakes is regulated by the influx of sulphur from the atmosphere,

Recent studies have shown that the oxidation rate for  $\text{SO}_2$  in the Copper Hill smelter plume is generally less than  $3\% \text{ h}^{-1}$  with  $1\% \text{ h}^{-1}$  as representative average<sup>5,13</sup>. The oxidation rate in the dispersed plume may be expected to be generally slower. The low oxidation rate implies that the isotopic effects associated with a rate controlled oxidation reaction such as

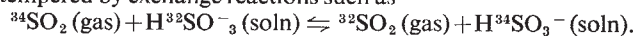


would be small. The  $\delta^{34}\text{S}$  for the residual  $\text{SO}_2$  should increase according to the relationship<sup>3</sup>:

$$\delta^{34}\text{SO}_2(\text{final}) - \delta^{34}\text{SO}_2(\text{initial}) = \beta(1-f)1000$$

where  $\beta$  is the fractional increase in the reaction constant for  $^{34}\text{SO}_2$  and  $(1-f)$  is the fraction of  $\text{SO}_2$  converted to  $\text{SO}_3$ .

Furthermore, the enrichment of  $^{34}\text{S}$  in the residual  $\text{SO}_2$  may be tempered by exchange reactions such as



It follows that near the smelter stack (100 km or so), the modification in the  $\delta^{34}\text{SO}_2$  will generally be small. Indeed, studies by Forrest and Newman<sup>13</sup> and J. O. N. and R. D. Coker (unpublished) show that the  $\delta^{34}\text{S}$  for the atmospheric  $\text{SO}_2$  is fairly constant within the 100 km radius of the stack. The homogeneous nature of the  $\delta^{34}\text{SO}_2$  around the stack implies that the  $\delta^{34}\text{S}$  data for lakes which derive their sulphur mostly from atmospheric source (notably the poorly buffered, shield lakes) will likewise be fairly uniform. This, indeed, is what we have observed. The exact  $\delta^{34}\text{S}$  value for the sulphur in these lakes obviously will depend on (1) the mechanisms of  $\text{SO}_2$  removal from the atmosphere, (2) the admixture of stack  $\text{SO}_2$  with the regional background  $\text{SO}_2$ , and (3) variations in the isotopic compositions of the ores being smelted (which may vary from +0.5 to +7.2‰, see Schwarcz<sup>14</sup>) and the operating

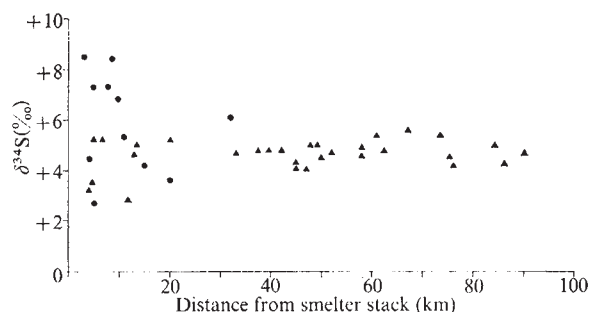


Fig. 3 Effect of distance from the stack on the isotopic composition of sulphur in the lake waters.

conditions of the plant. It is interesting to note that the  $\delta^{34}\text{S}$  data for these poorly buffered lakes are very similar to the mean annual  $\delta^{34}\text{S}$  values for bulk precipitation in the same region (J.O.N., unpublished results).

The weak correlation between pH and  $\delta^{34}\text{S}$  (Table 1) reflects the decoupling of the sulphur and proton cycles within these lakes. Unlike the conservative sulphate, protons are highly active and do not simply accumulate in lakes, presumably because of the synergistic influence of the atmospheric  $\text{CO}_2$ . This study thus illustrates the diagnostic and prognostic capabilities of  $\delta^{34}\text{S}$  measurements in polluted lakes, particularly when the pH and sulphate concentrations are also determined during study.

JEROME O. NRIAGU

Canada Centre for Inland Waters,  
Burlington, Ontario L7R 4A6,

HAROLD H. HARVEY

Department of Zoology, University of Toronto,  
Toronto, Ontario M5S 1A1, Canada

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## Coral-reef area and the contributions of reefs to processes and resources of the world's oceans

Do coral reefs constitute a quantitatively significant ecosystem on a global scale? The area, calcium mass balance, and fisheries yield of reefs provide important geographical, geochemical and biological criteria for answering this question. After considering them, I conclude that coral reefs should not be overlooked in the quantitative assessment of global resources.

Menard and Smith<sup>1</sup> updated hypsographic data for the world's oceans, and Professor Menard has kindly provided me with a summary of the areas within depth increments tabulated by 10° 'square' of latitude and longitude. The shallowest depth increment in that summary is 0–200 m. Wells<sup>2</sup> presented a map of the global distribution of coral reefs, updating earlier work. Wells' map was used to estimate visually (to the nearest 25%) the percentage of coastline and other shoals fronted by reefs within each 10° square. Menard and Smith's tabulation was used to determine the total area shallower than 200 m within those squares having reefs. I assumed that water depth is evenly distributed between 0 and 200 m and that along those coasts having reefs they extend from 0 to an average of 30 m water depth. Coral communities can extend well below 30 m, so this analysis should be conservative. Reef area within each square is thus estimated to be the product of 15% of the 0–200 m area times the proportion of shoal area with reefs.

This assessment of reef area includes not only the wave-resistant framework created by corals, coralline algae and other cementing organisms but also associated, contiguous and predominantly soft-bottom biological communities and sedimentary assemblages.

Table 1 summarises the results. Total reef area estimated according to this procedure is approximately  $6 \times 10^5$  km<sup>2</sup>, over half of which is in the Asiatic Mediterranean and Indian Oceans (see Fig. 1 and ref. 1 for the ocean boundaries used). As determined by comparison with the global hypsographic data<sup>1</sup>, reefs constitute about 0.17% of the world ocean area and about 15% of the shallow sea floor within the 0–30 m depth range. Newell<sup>3</sup> used earlier maps, arrived (by a method not explained in his text) at a global reef area of  $1.5 \times 10^6$  km<sup>2</sup>, and arbitrarily divided his estimates by 10. The average of his upper and lower area estimates is close to my estimate.

Calcification is obviously a major biogeochemical process on coral reefs, and a moderate amount is known about the CaCO<sub>3</sub> production rate of reefs. Comparison of the amount of CaCO<sub>3</sub> precipitated by reefs with the calcium input to the oceans provides a measure of the role of reefs in the world calcium balance. Many reef environments calcify at one of two characteristic rates<sup>4,5</sup>. A production rate of about 8 mol CaCO<sub>3</sub> m<sup>-2</sup> yr<sup>-1</sup> prevails over regionally extensive areas, whereas more localised and highly active portions of reefs calcify at 40 mol CaCO<sub>3</sub> m<sup>-2</sup> yr<sup>-1</sup>. By far the major proportional area of reefs calcifies relatively slowly

**Table 1** Area covered by the world's coral reefs, by major oceanic region

| Oceanic region<br>(abbreviation on Fig. 1) | Reef area<br>( $\times 1,000$ km <sup>2</sup> ) | % Total<br>reef area |
|--------------------------------------------|-------------------------------------------------|----------------------|
| Asiatic Mediterranean (ASM.)               | 182                                             | 30                   |
| Indian (IND.)                              | 146                                             | 24                   |
| South Pacific (S. PAC.)                    | 77                                              | 13                   |
| North Pacific (N. PAC.)                    | 76                                              | 12                   |
| Caribbean (CARIB.)                         | 57                                              | 9                    |
| North Atlantic (N. ATL.)                   | 32                                              | 5                    |
| Red Sea (RED)                              | 27                                              | 4                    |
| Persian Gulf (PER.)                        | 12                                              | 2                    |
| South Atlantic (S. ATL.)                   | 8                                               | 1                    |
| Total                                      | 617                                             | 100                  |

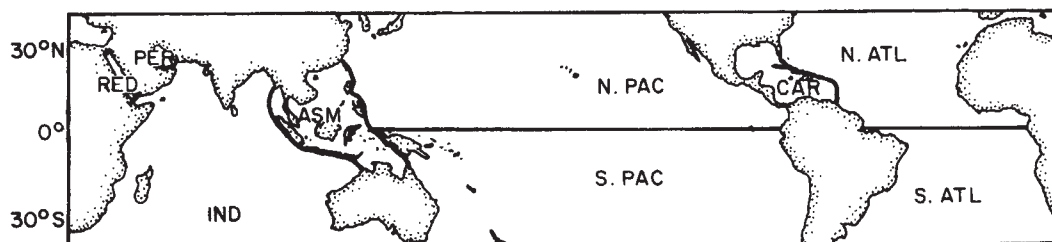
(for example, lagoons, sand flats, and so on), although the observer's attention may focus on areas with a high rate of calcification (for example, coral thickets, algal pavements, and so on). I estimate that 90–95% of the global reef area calcifies at the 'slow rate' and the remainder at the 'fast rate'; then reefs precipitate about  $6 \times 10^{12}$  mol CaCO<sub>3</sub> annually. This estimate is likely to be conservative. The proportion of reefs which calcifies at the higher rate may be substantially larger than I have estimated, and some authors<sup>6–8</sup> believe that certain estimates<sup>4</sup> of the upper modal rate of reef calcification are low. (I have deliberately omitted estimates strictly of vertical accretion rates, because of problems in intercalibrating vertical accretion and mass accretion data.) The most serious potential for error in this estimate is therefore in an underestimate of reef calcification (that is, that the high production area or rate has been underestimated).

The delivery of calcium to the world's oceans is about  $1.2 \times 10^{13}$  mol yr<sup>-1</sup> (ref. 9). Thus, reef production at least temporarily precipitates an amount of calcium equivalent to about 50% of the input. By the very construction of wave-resistant structures and sediment-trapping lagoons, reefs can be expected to retain a substantial fraction of their sedimentary products and to minimise dissolution. The quantitative importance of such re-solution remains unknown, but it seems clear that reef sediments constitute a quantitatively significant global sink for calcium.

Reef fisheries potential provides yet another indicator of the quantitative significance of coral reefs. It has been suggested that the potential of reef fisheries may be substantially larger than has generally been appreciated, because of operational problems in utilising that potential<sup>10</sup>. The reef fisheries of the Caribbean Sea and Western North Atlantic Ocean have been examined in some detail; the potential yield of demersal fishes from reefs and adjacent deep shelf areas has been estimated to be  $8 \times 10^8$  kg yr<sup>-1</sup> (ref. 11). I assume that the demersal fishes from deep shelf areas should be included with the reef fishes themselves in evaluating potential reef yield, because these deep shelf demersal fishes probably derive much of their nutrition from the reefs.

The Caribbean plus North Atlantic reefs comprise 14% of the world's reefs (Table 1). If one can validly extrapolate

**Fig. 1** Boundaries of oceanic provinces as used to evaluate reef areas.





from the fisheries potential of that area to the world's reefs, then world reef fisheries potential is about  $6 \times 10^9 \text{ kg yr}^{-1}$ . The present commercial oceanic fish landings are about  $7 \times 10^{10} \text{ kg yr}^{-1}$  (ref. 12); reef-related fisheries apparently have a potential yield near 9% of this total. Data are insufficient to establish what fraction of the reef fisheries potential is presently realised. Nor is it obvious to what extent subsistence fisheries excluded from the commercial landings might alter the importance of the reef fisheries potential.

These inventories are speculative and incomplete; nevertheless, they are sufficient to demonstrate that coral reefs are indeed a quantitatively significant ecosystem on a global scale.

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S. V. SMITH

University of Hawaii,  
Hawaii Institute of Marine Biology,  
P.O. Box 1346,  
Kaneohe, Hawaii 96744

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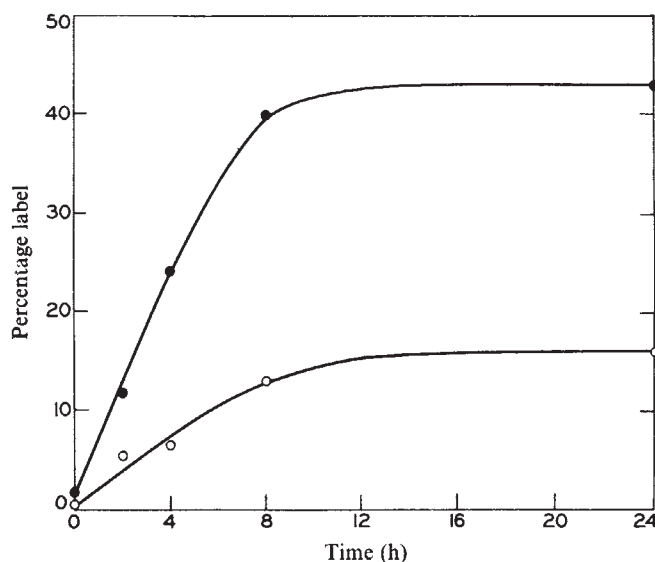
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## Production of methane and carbon dioxide from methane thiol and dimethyl sulphide by anaerobic lake sediments

METHANE THIOL (methyl mercaptan, MeSH), its oxidation product, dimethyl disulphide (DMDS), and dimethyl sulphide (DMS) are naturally occurring metabolites. They are also produced by paper mills using the kraft pulp process<sup>1</sup> and cause an industrial odour problem because of their low odour threshold. Although the biological production of these compounds is fairly well understood<sup>2</sup>, little is known about their biological decomposition. We report here that microbial populations present in anaerobic freshwater sediments and in anaerobic sewage digester sludge are capable of metabolising the carbon in these volatile organic sulphur compounds to methane and carbon dioxide. Therefore, anaerobic habitats may serve as sinks in the biogeochemical cycling of these compounds.

MeSH and DMDS are produced from the methiol group of methionine by both aerobic and anaerobic microorganisms<sup>3</sup>. DMS is usually a minor product of methionine breakdown, and is more often produced from sulphonium compounds, such as the thetins found in algae and fungi<sup>3</sup>. Lovelock *et al.*<sup>4</sup> detected DMS in ocean waters and in the atmosphere over soils and have suggested that DMS plays a major role in the atmospheric sulphur cycle, but further studies are needed before the role of DMS can be ascertained.

In our studies of anaerobic metabolism of methionine by Lake Mendota (Wisconsin) sediments using low levels of radiolabelled methionine, we have obtained evidence that MeSH was cleaved from methionine, and that this MeSH was subsequently metabolised to methane, carbon dioxide



**Fig. 1** Time course for  $^{14}\text{C}$ -methane (●) and carbon dioxide production (○) from  $^{14}\text{C}$ -labelled MeSH by Lake Mendota sediments. Five  $\mu\text{l}$  1 M NaOH containing 2.2 nmol (250,000 d.p.m.)  $^{14}\text{C}$ -labelled MeSH (New England Nuclear,) as the sodium mercaptide, was taken up into a Hamilton No. 701 10  $\mu\text{l}$  syringe, and then 5  $\mu\text{l}$  1 M HCl was also taken up into the same syringe. This mixture was immediately injected through a neoprene serum septum into a 10 ml serum vial containing 1 ml lake sediment in a nitrogen atmosphere. Thus, the solution injected was  $^{14}\text{C}$ -MeSH in 10  $\mu\text{l}$  1 M NaCl. The sediments were mixed with a vortex mixer and incubated at  $15^\circ\text{C}$ , the highest *in situ* temperature reached by the sediments. Preliminary experiments showed that the  $^{14}\text{C}$ -MeSH was rapidly absorbed by the sediment, although some was also absorbed into the neoprene septum. The neoprene septum was necessary to keep the sediments anaerobic. Production of  $^{14}\text{C}$ -labelled methane and carbon dioxide was followed using the gas chromatographic-proportion counter method of Nelson and Zeikus<sup>5</sup>.

and hydrogen sulphide<sup>5</sup>. For this mechanism to be possible, the metabolism of MeSH to these products needed to be demonstrated. Also, because metabolism by anaerobic sediments may be an important sink for DMS, it was of interest to determine if similar products were formed from DMS. The degradation of these compounds by Lake Mendota sediments was followed using  $^{14}\text{C}$ -labelled MeSH and DMS. (For details, see Fig. 1.)

As shown in Fig. 1,  $^{14}\text{C}$ -MeSH was rapidly metabolised to methane and carbon dioxide by the lake sediments. The ratio of methane to carbon dioxide produced is close to the 3:1 ratio predicted if MeSH was fermented in the manner predicted by the equation:



This is similar to methanol fermentation by *Methanosarcina barkeri*<sup>7</sup>.

One indication that a reaction is biological is that the reaction shows a temperature optimum. Figure 2 shows that MeSH metabolism showed a definite optimum at  $37^\circ\text{C}$ , and there was essentially no activity at  $55^\circ\text{C}$ . Although the  $37^\circ\text{C}$  optimum is much higher than the maximum *in situ* temperature reached by the sediments ( $15^\circ\text{C}$ ), it has been shown by Zeikus and Winfrey<sup>8</sup> that methanogenesis in Lake Mendota sediments has a similar optimum. At temperatures higher than  $15^\circ\text{C}$ , the ratio between methane and  $\text{CO}_2$  from MeSH increased, possibly due to a shift in metabolism caused by increased hydrogen production by sediment populations at these temperatures<sup>9</sup>.

The effects of various additions on MeSH metabolism by sediments are presented in Table 1. Chloroform, a well-known inhibitor of one-carbon metabolism<sup>10</sup>, when added to sediment at a fairly low concentration prevented all

methane production from MeSH and allowed a small amount of carbon dioxide production. Incubating the sediments in a hydrogen atmosphere greatly increased the ratio of methane to carbon dioxide and caused greater overall metabolism of  $^{14}\text{C}$ -MeSH. Sulphate, shown by Winfrey and Zeikus<sup>9</sup> to inhibit methanogenesis from bicarbonate and acetate in Lake Mendota sediments, was found not to affect metabolism of MeSH. If the sediments were heated to 70 °C for 1 h in a nitrogen atmosphere before incubation with labelled MeSH, although there was no visible change in the sediments, there was no activity. Sediment heated to 70 °C thus could serve as a killed control. Anaerobic sewage digester sludge (Madison Metropolitan Sanitary District) was also tested for its ability to metabolise MeSH and was found to produce almost exclusively methane, most likely because of the large concentrations of hydrogen donors in the digester sludge.

Figure 3 shows that  $^{14}\text{C}$ -labelled DMS (20,000 D.P.M., 2.2 nmol) added to the headspace over 1 ml sediment, was also metabolised to methane and carbon dioxide, although the ratio of these was greater than 3 : 1. As shown in Table 1, the effects of various additions on  $^{14}\text{C}$ -DMS metabolism were similar to those described for  $^{14}\text{C}$ -MeSH metabolism. In other studies<sup>5</sup>, we have found that these additions had similar effects on the metabolism by sediments of  $^{14}\text{C}$ -methionine labelled in the terminal methyl group.

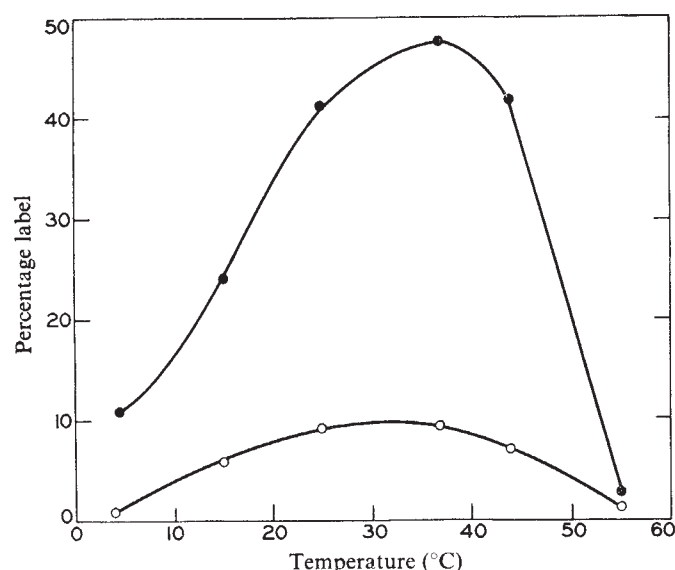


Fig. 2 Effect of temperature on  $^{14}\text{C}$ -methane (●) and carbon dioxide production (○) from  $^{14}\text{C}$ -labelled MeSH by Lake Mendota sediments. Sediments were incubated with the label for 4 h in a nitrogen atmosphere in 10-ml serum vials capped with neoprene serum septa.

The most likely candidates for the metabolism of MeSH and DMS in the sediments are the methanogenic bacteria, especially as there is some resemblance between volatile organic sulphur compounds and coenzyme M (2-mercaptosulphanesulphonic acid), a methyl group carrier used by methanogenic bacteria<sup>11</sup>. The free energy for the fermentation of MeSH is -8.3 kilo calories/mol MeSH, whereas the free energy for the reduction by hydrogen of MeSH to methane and hydrogen sulphide is -16.5 kilo calories/mol MeSH<sup>12,13</sup>; so either reaction is energetically favourable and may generate enough energy for synthesis of adenosine triphosphate and therefore support microbial growth<sup>12</sup>. The free energies for DMS metabolism are similar. In preliminary experiments, we have found no production of methane or carbon dioxide from either  $^{14}\text{C}$ -MeSH,  $^{14}\text{C}$ -DMS

Table 1 Effects of various additions on methane and carbon dioxide production from  $^{14}\text{C}$ -labelled MeSH and DMS by Lake Mendota sediments

| Sample                                       | Percentage label                         |                 |                                          |                 |
|----------------------------------------------|------------------------------------------|-----------------|------------------------------------------|-----------------|
|                                              | $^{14}\text{C}$ MeSH*<br>CH <sub>4</sub> | CO <sub>2</sub> | $^{14}\text{C}$ -DMS†<br>CH <sub>4</sub> | CO <sub>2</sub> |
| Control                                      | 26                                       | 8               | 28                                       | 3               |
| CHCl <sub>3</sub> (750 µg ml <sup>-1</sup> ) | 0                                        | 2               | 0                                        | 0               |
| H <sub>2</sub> (1 atm)                       | 48                                       | 2               | 45                                       | 0               |
| SO <sub>4</sub> <sup>2-</sup> (10 mM)        | 30                                       | 8               | 35                                       | 7               |
| Heat killed (70 °C, 1 h)                     | 0                                        | 0               | 0                                        | 0               |
| Digester sludge‡                             | 48                                       | 2               | ND                                       | ND              |

ND, Not determined.

\*2.1 nmol (250,000 d.p.m.) added to 1 ml sediment. The sediments were incubated with label for 4 h at 15 °C in a nitrogen atmosphere in 10-ml serum vials capped with neoprene serum septa.

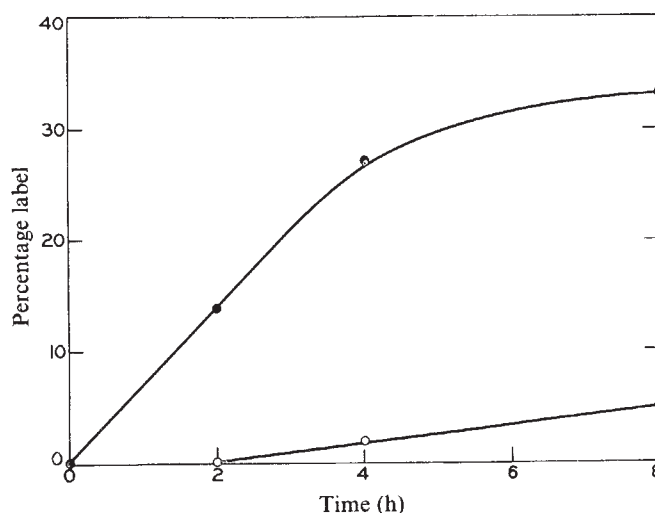
†2.2 nmol (20,000 d.p.m.) added to 1-ml sediments. Conditions as for MeSH.

‡Anaerobic sewage digester sludge (Madison Metropolitan Sewage District) was handled in the same manner as lake sediments except that it was incubated at 37 °C.

or methyl labelled  $^{14}\text{C}$ -L-methionine by cultures of *Methanobacterium ruminantium*, *Methanobacterium thermoautotrophicum*, or *Methanosarcina barkeri* (methanol fermenting). It is possible that the physiological conditions were not correct for the organisms to carry out these reactions. Also, enrichment cultures for organisms which metabolise these compounds have failed, so that the organisms in the sediment which carry out volatile organic sulphur compound metabolism in the sediments are presently unknown.

Although sulphur was not followed in these experiments, our studies using  $^{35}\text{S}$ -labelled methionine<sup>5</sup> have shown that hydrogen sulphide was the major product of methionine degradation, and as it is likely that methionine breaks down

Fig. 3 Time course for  $^{14}\text{C}$ -methane (●) and carbon dioxide production (○) from  $^{14}\text{C}$ -labelled DMS (2.1 nmol ml<sup>-1</sup>, 20,000 d.p.m.) by Lake Mendota sediments. Conditions were as in Fig. 1.  $^{14}\text{C}$ -labelled DMS was prepared by bacterial reduction of  $^{14}\text{C}$ -labelled dimethyl sulphoxide DMS (NEN, 4.2 mCi nmol<sup>-1</sup>). We have found in other studies (unpublished) that *Escherichia coli* will reduce dimethyl sulphoxide to DMS with no other product formed. The  $^{14}\text{C}$ -dimethyl sulphoxide was added to growth medium (5g l<sup>-1</sup> each of tryptone, yeast extract and glucose) inoculated with *E. coli*. The culture was incubated in a nitrogen atmosphere at 30 °C for 24 h. After incubation, the pH of the medium was increased to 11 to absorb the CO<sub>2</sub> and organic acids produced by the culture. The  $^{14}\text{C}$ -DMS was removed from the head space using a gas-tight syringe. The purity of the radiochemicals used was verified using a Packard 419 gas chromatograph (Packard, fitted with a flame photometric detector sensitive to sulphur compounds).





by way of MeSH, it can be inferred that the sulphur of MeSH was also converted to sulphide.

We have been unable to detect any volatile sulphur compounds besides hydrogen sulphide in Lake Mendota or the waters overlying them, even if cryogenic trapping techniques were used, in agreement with our findings that these compounds are rapidly metabolised by sediments. We have detected 1–10 ng l<sup>-1</sup> DMS in aerobic surface waters of Lake Mendota during summer months when a dense cyanobacterial (blue-green algal) bloom was present. The sediments in contact with these waters in shallow parts of the lake may be a sink for DMS. It is possible that other shallow water sediments and marsh sediments are also sinks for DMS.

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STEPHEN H. ZINDER\*  
T. D. BROCK

Department of Bacteriology,  
University of Wisconsin,  
Madison, Wisconsin 53706

\*Present address: Department of Earth and Space Science, UCLA, Los Angeles, California 90024.

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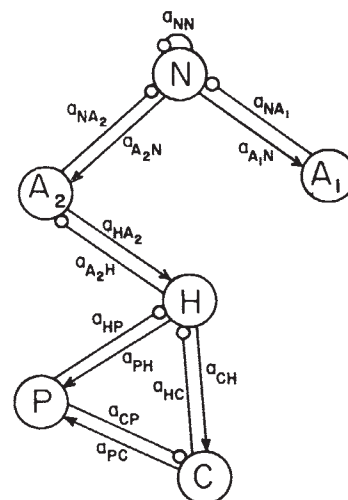
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## Cybernetic mechanisms in lake plankton systems: how to control undersirable algae

Toxic algal blooms represent a serious nuisance in lakes and ponds. Not only do they form floating scums and impart odours and tastes to the water, but they are also associated with the occurrence of dermatitis in our own species and fatal poisoning in livestock. To reduce algal levels, man resorts to various practices that range from chemical 'control' to artificial mixing of the lake<sup>1</sup>. Most of these have deleterious, if not immediately toxic, effects on the other compartments of the system as well. In this report safer alternatives are presented.

As toxicity is primarily a defence mechanism evolved against herbivores, the problem of controlling toxic algae can be transposed to the simpler one of controlling inedible algae. A simple loop model of a lake plankton community containing an inedible compartment is then sufficient to reveal how the system will respond to perturbations introduced at different trophic levels. This, in turn, suggests various ways to achieve biological control of the inedible (undesirable) species.

The model considered (Fig. 1a) is a simplified planktonic food web reduced to six variables: nutrients (N), inedible algae (A<sub>1</sub>), edible algae (A<sub>2</sub>), herbivores (H), carnivores (C) and planktivorous predators (P). The distinction between edible and inedible algae is based on the overwhelming evidence<sup>2–8</sup> that phytoplankton cells are selected on the basis of size, taste and morphology.



|            |             |             |             |           |           |
|------------|-------------|-------------|-------------|-----------|-----------|
| $-a_{NN}$  | $-a_{NA_1}$ | $-a_{NA_2}$ | 0           | 0         | 0         |
| $a_{A_1N}$ | 0           | 0           | 0           | 0         | 0         |
| $a_{A_2N}$ | 0           | 0           | $-a_{A_2H}$ | 0         | 0         |
| 0          | 0           | $a_{HA_2}$  | 0           | $-a_{HC}$ | $-a_{HP}$ |
| 0          | 0           | 0           | $a_{CH}$    | 0         | $-a_{CP}$ |
| 0          | 0           | 0           | $a_{PH}$    | $a_{PC}$  | 0         |

Fig. 1 a, Loop diagram of the six-variable system. Positive effect of variable  $i$  on variable  $j$  is indicated by an arrow going from  $i$  to  $j$ . Negative effect is indicated by a circle. Nutrient resources are not self-reproducing and are thus self-damped. b, Matrix of the system, taking all the  $a_{ij}$  as positive numbers and representing the directions of their effects by the sign in front.

Here the term 'inedible' is used realistically: we recognise the likelihood of some members of group A<sub>1</sub> being occasionally ingested by grazers but consider these instances truly exceptional in a community in which 'edible' algae are also present. Accordingly, throughout this report A<sub>1</sub> refers to an heterogeneous group of species including all blue-green algae, irrespective of size, plus all other phytoplankton species larger than 50  $\mu$ m, but excluding the common diatom *Asterionella formosa*. As the latter is easily broken down and ingested<sup>6,8</sup> it was integrated in A<sub>2</sub>. Blue-greens excepted, all algae smaller than 50  $\mu$ m were considered edible. The few taxa possessing an ambiguous trophic status were excluded from either group. This applied to the gelatinous greens *Sphaerocystis schroeteri* and *Elakatothrix gelatinosa* that may be enhanced by grazers<sup>9</sup>, the colonial diatoms *Fragilaria* and *Tabellaria* that can be ingested by copepods when fragmented (K. Porter, personal communication) and the colonial chrysomonad *Dinobryon* that may be a major food source for daphnids when it is present in high density<sup>10</sup>.

The dynamics of this planktonic model can be readily explored by loop analysis. This technique recently developed by Levins<sup>11,12</sup> is particularly applicable to ecological systems in which most interactions among the biological variables can only be specified qualitatively. Loop analysis is based on the equivalence between differential equations near equilibrium and matrices and their loop diagrams. Thus, in our particular system the elements  $a_{ij}$  of the matrix and the loop diagram (Fig. 1) represent the effect of variable  $j$  on the growth of variable  $i$  when the equation  $dX_i/dt = f_i(X_1, \dots, X_j, \dots, X_n)$  is solved at equilibrium. As all stability require-

ments are satisfied for our model, one can assess mathematically the consequences on the entire system of changes in each variable. The reader interested in the details of the technique is referred to the formal treatment of loop analysis theory<sup>12</sup>.

As shown in Table 1, the predictions derived from our model are often far from obvious. For example, increasing predator density (case 6) will leave the herbivore level unaltered yet increase the inedible algae. Also the addition of herbivores to the system (case 4) has no effect on the standing stock of all animal consumers. Thus, one cannot infer the response of one variable to changes in another from the simple knowledge of the direct link between the two. Clearly the structure of the entire network needs to be taken into account, as its effects may regularly overpower simple-link effects. This is where the power of loop analysis lies and why it often yields unexpected results<sup>12,13</sup>, especially when there is no direct link between the two variables considered. For instance we can predict in this manner that adding inedible algae to our system (case 2) will increase the carnivore level but will reduce the standing stock of the other animal consumers.

As the evolution of inedibility in algae leads to the development of toxicity that is considered undesirable by man, we can treat  $A_1$  as the variable to be reduced. According to our model, this can be achieved in four ways (see column  $A_1$  in Table 1): that is, either by reducing the rate of nutrient supply, by increasing carnivore levels or by reducing predator or herbivore stocks.

The first method is particularly applicable when there is a point discharge of nutrients into the water body: this was demonstrated spectacularly by the diversion of sewage from Lake Washington<sup>14</sup>. Unfortunately nutrient inputs are not controllable in most cases. Biological manipulation of lake communities may then well represent the only means for selective algal control. Although this approach has been convincingly advocated by Shapiro *et al.*<sup>15</sup> it has remained surprisingly neglected. The results of our work suggest simple ways to achieve biological control of undesirable algae. Among these, the introduction of herbivore-specific disease or the addition of primary carnivores would seem particularly worth trying.

It is clear that the table of predictions generated by our model would take years to be tested in its entirety. Its general validity, however, is supported by long-term field experiments and indirect evidence from previously published work. A particularly well documented case, because of the great concern for the consequences of artificial eutrophica-

tion, is that of nutrient addition. We predict here (case 1), in agreement with previous models of nutrient enrichment<sup>13,16,17</sup>, that the increment in nutrients is picked up entirely by the inedible algae. Evidence from 'cultural' eutrophication<sup>14,18</sup> and fertilisation experiments<sup>19</sup> supports our contention. The common response of lakes to nutrient enrichment is an immediate increase in the density of the algae considered inedible and a change in the qualitative composition, but not in the standing stock, of the consumer levels. Further, as  $N$  itself remains unaltered, it can be understood why nutrient levels are poor indicators of eutrophication<sup>20</sup>.

Conversely, we predict that a decrease in the rate of nutrient input (case 7) will reduce only the level of inedible algae without altering those of  $N$ ,  $A_2$ ,  $H$ ,  $P$  or  $C$ . This would indicate, as already pointed out by Phillips<sup>16</sup> for marine systems, that inedible algae serve as a buffer against variations in the nutrient supply. This hypothesis was tested by following from June to November 1976 the effects on lake plankton of cutting the nutrient input from the sediments. The communities studied were enclosed in a series of polyethylene tubes, each 1 m in diameter and 10 m deep, suspended in Heney Lake, a lake with low levels of nutrients 60 miles north of Ottawa. Community structure was monitored by weekly sampling at 0, 3, 6 and 9 m, followed by microscopic enumeration using the sedimentation technique of Utermöhl<sup>21</sup>.

Our predictions (case 7) were all confirmed. Comparing tubes open at both ends and sunk in the sediments with tubes sealed at the bottom revealed that their population stocks were similar at each trophic level throughout the 5-month study (Wilcoxon matched-pairs signed-ranks test) except for the inedible algae that were significantly reduced ( $P < 0.025$ ) in the latter case. In light of this 'enclosure effect' and of the increasing popularity of this technique among aquatic ecologists, the use of tubes in contact with the sediments is recommended as the closest simulation of lake conditions.

Support for our predictions concerning the response of planktonic communities to changes in predation levels (cases 6 and 9) is found in the classic investigations by Hrbáček<sup>22,23</sup>. He documented a shift from nanoplankton to netplankton and a change in the species composition but not in the standing crop of the herbivores after increases in stocks of planktivorous fish. Then, by eliminating fish stocks altogether he was further able to induce a shift from netplankton to nanoplankton. More recently Smyly<sup>24</sup> detected no noticeable change in herbivore levels following the reduction in fish predation in experimental enclosures.

The response of plankton systems to reduction in both herbivore and carnivore levels in polyethylene tubes at Heney Lake was also investigated. All components of the planktonic community were represented in our experimental enclosures, including, in addition to 99 phytoplankton species, herbivores such as *Bosmina*, *Chydorus*, *Daphnia* and *Diaptomus*, the primary carnivores *Epischura* and *Mesocyclops*, and the predators *Chaoborus* and *Leptodora*. Throughout the 5-month study  $H$  and  $C$  levels were reduced each week by passing a net of 0.75 m in diameter and 135  $\mu\text{m}$  mesh aperture through the tubes.

Examination of the loop diagram (Fig. 1) shows that the net outcome of decreasing jointly  $H$  and  $C$  levels is to reduce the carnivore population alone, because of the negative  $CH$  loop. The consequent predictions (case 8) were generally verified by our experiments. Using unperturbed enclosures for comparison, we found that the manipulations resulted in a significant increase ( $P < 0.025$ ) in the biomass of inedible algae and in no significant changes in herbivore and carnivore levels (Wilcoxon test). The only discrepancy was that no significant decrease in edible algae was recorded.

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**Table 1** Predicted response of plankton systems to perturbations in each variable

| Case | Perturbation* | Effect on the level of      | Predictions supported by                                                           |  |  |  |  |
|------|---------------|-----------------------------|------------------------------------------------------------------------------------|--|--|--|--|
|      |               | $N$ $A_1$ $A_2$ $H$ $C$ $P$ |                                                                                    |  |  |  |  |
| 1    | $+N$          | 0 + 0 0 0 0 0               | Fertilisation experiments and evidence from 'cultural' eutrophication (refs 16-18) |  |  |  |  |
| 2    | $+A_1$        | - 0 0 - + -                 | (Currently being tested in long-range experiments <i>in situ</i> )                 |  |  |  |  |
| 3    | $+A_2$        | 0 0 0 + - +                 |                                                                                    |  |  |  |  |
| 4    | $+H$          | 0 + - 0 0 0                 |                                                                                    |  |  |  |  |
| 5    | $+C$          | 0 - + 0 0 +                 |                                                                                    |  |  |  |  |
| 6    | $+P$          | 0 + - 0 - 0                 | Lake manipulations (ref. 21)                                                       |  |  |  |  |
| 7    | $-N$          | 0 - 0 0 0 0                 | Enclosure experiments of the authors                                               |  |  |  |  |
| 8    | $-C$          | 0 + - 0 0 -                 |                                                                                    |  |  |  |  |
| 9    | $-P$          | 0 - + 0 + 0                 | Lake manipulations (ref. 22)                                                       |  |  |  |  |

\* Three cases ( $-A_1$ ,  $-A_2$ ,  $-H$ ) have been omitted to avoid redundancy. They can be derived simply by reversing the sign of the effect of  $+A_1$ ,  $+A_2$ , and  $+H$ , respectively.  
+, Enhancing effect; -, inhibiting effect; 0, no effect.



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FRÉDÉRIC BRIAND  
EDWARD MCCAULEY

Department of Biology,  
University of Ottawa,  
Ottawa, Canada K1N 6N5

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## Calcium-dependent sensory-motor response of a marine dinoflagellate to CO<sub>2</sub>

EXTERNAL calcium levels have been shown to have an important role in the regulation of sensory responses of freshwater protozoa<sup>1,2</sup>. Such responses in many species seem to be associated with transient changes in calcium permeability of the cell membrane, which lead to action potentials that in turn seem to modify swimming behaviour. In view of the many intriguing similarities of such phenomena to events observed in neurones, it is of interest to establish whether marine protozoa, living in a high sodium environment, exhibit sodium- or calcium-dependent behaviour. We report here calcium-dependent sensory-motor behaviour in the marine dinoflagellate, *Cryptocodinium cohnii*.

The response studied is of a type usually termed 'aerotaxis' and has been observed in many species of protists<sup>3,4</sup>. In this, cells are seen to aggregate in zones parallel to the edge of a coverslip or around the border of bubbles trapped under the coverslip; aggregates around bubbles disperse with time, but those near the coverslip edge persist for long periods if drying is prevented. These dynamic aggregates have usually been thought to be due to behavioural responses to oxygen gradients within the medium. In the heterotrophic dinoflagellate *C. cohnii*, however, our evidence indicates that aggregation occurs in response to gradients of CO<sub>2</sub> rather than of O<sub>2</sub>.

Figure 1a and b shows typical aggregations of *C. cohnii* about a bubble and at the coverslip edge. Observations of swimming paths of individual organisms moving away from or towards the air-medium interface indicate that the latter have much larger mean free paths than the former, which are comparable to swimming paths in regions away from the

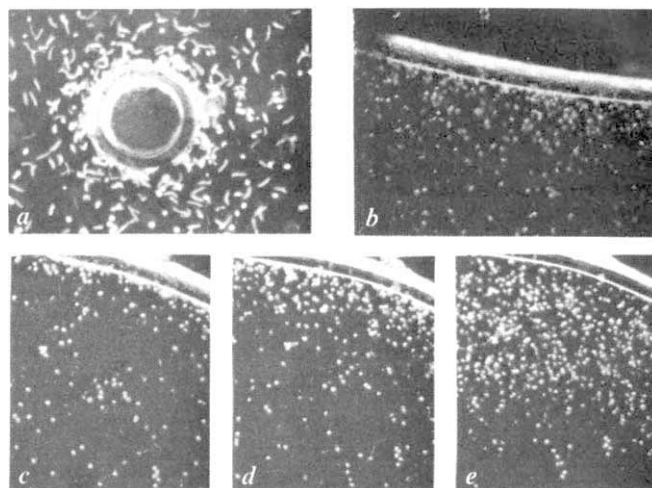


Fig. 1 a, *Cryptocodinium cohnii* aggregating about a bubble of air. b, Typical aggregation at meniscus at edge of coverslip. c-e, Effects of exposure to gentle stream of CO<sub>2</sub>. c, Aggregation at coverslip edge just before exposure, d, Cells starting to move from the interface as CO<sub>2</sub> reaches it. e, About 1 s later. Exposure times: a, 1/4 s; b-e, 1/16 s. Scale is given by bubble (a) which has 0.2 mm diameter.

interface; Table 1 shows typical data. Thus, the aggregation is not due to forces such as surface tension, but is a true behavioural response.

When preparations are placed in closed containers and the containers are thoroughly flushed with many times their volume of N<sub>2</sub> gas, the aggregation response remains unaffected, suggesting that an oxygen gradient is unlikely to be the cue for this behaviour. Furthermore, non-lethal concentrations of several respiratory poisons—sodium cyanide, oligomycin, sodium amytal, sodium azide, 2,4-dinitrophenol and salicylhydroxamic acid—had no discernible effect on aggregation by this facultative anaerobe. The reducing agents dithiothreitol, glutathione and tetrazolium chloride were also without effect at nonlethal levels.

When preparations were exposed to a gentle stream of CO<sub>2</sub> or CO<sub>2</sub>-enriched air, however, the aggregating cells immediately swam away from the interface (Fig. 1c-e). This response was quite dramatic and reproducible; control experiments with HCl vapour indicated no comparable response to severe pH changes, so this is apparently a true

Table 1 Movements of *C. cohnii* around air-medium interface

| A, Distance travelled toward air bubble before turning (mm):     |                    |           |           |           |           |
|------------------------------------------------------------------|--------------------|-----------|-----------|-----------|-----------|
|                                                                  | 0–0.37             | 0.37–0.74 | 0.74–1.11 | 1.11–1.48 | 1.48–1.85 |
|                                                                  | (No. of organisms) |           |           |           |           |
| Expt 1                                                           | 3                  | 3         | 7         | 5         | 32        |
| Expt 2                                                           | 1                  | 0         | 1         | 4         | 7         |
| Expt 3                                                           | 4                  | 5         | 3         | 4         | 20        |
| B, Distance travelled away from air bubble before turning:       |                    |           |           |           |           |
| Expt 1                                                           | 18                 | 14        | 1         | 2         | 1         |
| Expt 2                                                           | 6                  | 2         | 3         | 1         | 0         |
| Expt 3                                                           | 30                 | 3         | 3         | 2         | 0         |
| C, Distance travelled in a region with no bubble before turning: |                    |           |           |           |           |
| Expt 1                                                           | 34                 | 7         | 1         | 0         | 0         |
| Expt 3                                                           | 27                 | 5         | 1         | 0         | 0         |

Individual cells were observed from the beginning of a straight swimming path to the point of first turning; distance was obtained by observing the number of units of an ocular micrometer spanned by the swimming path. Aggregation was less pronounced in experiment 2 than in experiments 1 and 3. Similar data were obtained for cells swimming towards and away from the coverslip edge as for cells swimming towards and away from a bubble.

CO<sub>2</sub> response. After prolonged incubation in a CO<sub>2</sub>-enriched atmosphere, cells became uniformly distributed under the coverslip, with no tendency to aggregate, but seemed to behave normally in other respects. We conclude that aggregation is a response to a gradient in CO<sub>2</sub>, produced by the cell's metabolism, rather than to an O<sub>2</sub> gradient. We found that aggregation occurs even in bicarbonate-saturated media, and so is presumably due to a gradient caused by diffusion of dissolved CO<sub>2</sub> near the meniscus rather than to some other ionic species; aggregation was observed over a pH range of 5.5–8.0, although most work was done with buffered media at pH 6.2–6.4.

Cells washed by gentle centrifugation, resuspended in 3% NaCl solution, seemed to have normal motility but did not aggregate or respond to CO<sub>2</sub> exposure. Mean free paths of swimming organisms in Ca-free medium were comparable with those in normal (Ca-containing) media in regions away from the air-medium interface (that is, regions of no aggregation), as shown in Table 2. The response was seen, however, when calcium at  $\geq 0.5$  mM was added as CaCl<sub>2</sub>, CaSO<sub>4</sub>, or calcium succinate. When an excess of the precipitating Ca-binder oxalic acid was added, the response disappeared again, but it could be restored by addition of more Ca. Of many cations tested, only Sr<sup>2+</sup> (as SrCl<sub>2</sub>) could replace Ca. Mn (MnSO<sub>4</sub>), Zn (ZnSO<sub>4</sub>), Ba (BaCl<sub>2</sub>·2H<sub>2</sub>O), Co (CoSO<sub>4</sub>) and Ni (NiSO<sub>4</sub>) were ineffective in restoring the response.

**Table 2** Dependence of *C. cohnii* movement on Ca<sup>2+</sup>

*A*, Distance travelled before turning by washed cells resuspended in 3% NaCl (mm):

|  | 0–0.37             | 0.37–0.74 | 0.74–1.11 | 1.11–1.48 | 1.48–1.85 |
|--|--------------------|-----------|-----------|-----------|-----------|
|  | (No. of organisms) |           |           |           |           |

Towards bubble:

|        |    |   |   |   |   |
|--------|----|---|---|---|---|
| Expt 1 | 17 | 2 | 2 | 0 | 2 |
| Expt 2 | 20 | 2 | 1 | 0 | 1 |

Away from bubble:

|        |    |   |   |   |   |
|--------|----|---|---|---|---|
| Expt 1 | 18 | 5 | 1 | 0 | 0 |
| Expt 2 | 18 | 3 | 2 | 0 | 0 |

In region not near bubble:

|        |    |   |   |   |   |
|--------|----|---|---|---|---|
| Expt 1 | 16 | 1 | 0 | 1 | 0 |
| Expt 2 | 11 | 3 | 0 | 1 | 0 |

*B*, Distance travelled before turning by washed cells after addition of CaCl<sub>2</sub> 1 mM:

|                 |   |   |    |    |    |
|-----------------|---|---|----|----|----|
| Towards bubble: |   |   |    |    |    |
| Expt 1          | 5 | 5 | 10 | 16 | 17 |
| Expt 2          | 2 | 6 | 8  | 4  | 13 |

Away from bubble:

|        |    |   |   |   |   |
|--------|----|---|---|---|---|
| Expt 1 | 30 | 5 | 1 | 1 | 0 |
| Expt 2 | 24 | 4 | 2 | 0 | 0 |

This calcium dependence in behaviour of a marine protist may reflect a fundamental role of Ca in sensory transduction; this might also explain its use as a transducer or messenger in a wide variety of metazoan preparations. (Calcium has been implicated in the action potential of *Aplysia* neurones<sup>5,6</sup>, in various potentials in the vertebrate central nervous system<sup>7,8</sup>, in action potentials of anterior pituitary and other endocrine cells<sup>9</sup>, as a second messenger in glycogen phosphorylase induction in liver cells<sup>10</sup>, in the activation of sea urchin eggs<sup>11</sup>, and in exocytosis in the adrenal medulla and elsewhere<sup>12</sup>.)

Some behavioural effects of CO<sub>2</sub> in protozoa have been reported. Invasion of host cells by the parasite *Toxoplasma gondii* is enhanced by CO<sub>2</sub> (ref. 13). In *Chlamydomonas* the light threshold for reversal of phototaxis (the

inversion level) is greatly influenced by CO<sub>2</sub> levels<sup>14</sup>. In *C. cohnii* the adaptive value of the CO<sub>2</sub> response is not yet clear. This species is a saprophyte, typically found in samples of rotting *Fucus* and other seaweeds<sup>15</sup>, and so the response may serve as a dispersal mechanism for swimmers from one substrate to another.

Previous reports described behavioural responses of *C. cohnii* to agar gels impregnated with traces of chemicals that might serve as ecological cues<sup>16</sup>, or with certain neurohumors and related molecules<sup>17,18</sup>. In addition to the aggregation discussed here, there is another type of dynamic clumping often seen in older cultures, and apparently associated with mating<sup>19</sup>; the presumed pheromone involved in this latter is not yet identified however.

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D. C. R. HAUSER  
D. PETRYLAK  
G. SINGER  
M. LEVANDOWSKY

Haskins Laboratories of Pace University,  
41 Park Row, New York, New York 10038

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## Recovery, behaviour and evolutionary implications of live Monoplacophora

THE Monoplacophora are molluscs with bilateral serially repeated structures<sup>1</sup>. Their shells occur among the oldest mineralised hard parts in the early Cambrian, and because they are traceable from there only to the mid-Devonian they had been considered extinct<sup>2,3</sup>. However, in 1957 Lemche<sup>4</sup> reported an extant species in the deep sea. By 1972 six further extant species and several taxonomically unassigned specimens had become known from abyssal depths<sup>5–10</sup>, showing that the Monoplacophora continued to exist in post-mid-Devonian time in the deep sea. These finds also established that such molluscs are widely distributed on the deep-sea floor<sup>5–10</sup>. Two specimens were obtained about 1,800 m deep, but all others came from between 3,000 and 6,500 m (refs 5–10). McLean has reported a new, minute species obtained from cobbles at depths of 174, 229 and 388 m on the Cortes Ridge off southern California<sup>11,12</sup>. He is assigning it to the genus *Vema*. This is the first record of an extant monoplacophoran which, like early Palaeozoic species, inhabits shallow bathyal depths and, like some Silurian species, occurs on a hard substrate<sup>13</sup>. I report here the recovery of live specimens of this monoplacophoran.

In February, July and November 1977, I obtained 16 live specimens of McLean's *Vema* sp. on board the RV Velero



IV with a boxcorer on the Cortes Ridge off southern California ( $32^{\circ}58'12''\text{N}$ ;  $119^{\circ}32'38''\text{W}$  to  $33^{\circ}04'\text{N}$ ;  $119^{\circ}34'\text{W}$ ) at depths from 348 to 402 m. The monoplacophorans were attached to phosphorite nodules which occur in various densities at these sites (Fig. 1). Individuals varied from 1.2 mm to 2.1 mm long. Bottom water temperatures ranged from  $7.3^{\circ}\text{C}$  in winter to  $12.5^{\circ}\text{C}$  in summer. Specimens with their nodule substrate were kept in Petri dishes placed in tanks of water maintained at  $8.4\text{--}8.5^{\circ}\text{C}$ .

The animals stayed alive for up to 25 d. I observed them by placing the Petri dishes from the tanks under a binocular microscope. Strong incandescent light induced defensive behaviour similar to that of patellacean gastropods: an animal raised itself high above the foot, then tilted the shell either sideways or more commonly anteriorly, and thereafter rotated the shell in alternate directions sideways, as far as  $90^{\circ}$  from the body axis at rest. Movements of the shell were accompanied by acceleration in the beating of the gills. The animals then moved slowly to more shaded areas of the cobbles. Because of strong heat from the lights, the water temperature rose during observations and it was not clear whether the defensive behaviour and flight was in part a response to increased temperature. The animals were therefore observed under infrared and ultraviolet-filtered light where the basic behaviour patterns remained the same even though the water temperature increased only slightly. The only difference was a noticeable reduction in gill movement. This suggests that this *Vema* sp. is photonegative.



Fig. 1 Phosphorite nodules covering the sea floor where two live monoplacophorans were recovered by the boxcorer to which the camera was attached. Note sediments between and in surface depressions of nodules. The depth was 381 m.

This animal can stretch its foot to raise the shell as far as the height of the shell. Even at a slight elevation the foot becomes visible. Its distal part is invariably expanded (Fig. 2a). With further elevation of the shell the foot undergoes marked morphological changes. Initially the musculature forms broad longitudinal columnar bulges (Fig. 2b). Thereafter, a constriction develops between the distally expanded base and the proximal segment, where individual pedal muscle strands become superficially discernable (Fig. 2c). The ivory coloured foot is speckled with dark pigmented spots (Fig. 2a) and shows a dark reddish brown patch posteriorly below the anus (Fig. 2d). The distal finger-tip shaped ends of several gill branches were repeatedly seen below the shell margin (Fig. 2a). An anteriorly inclined pigmented structure, apparently tentacular in shape can be seen between the foot and the shadowy outline of the head (Fig. 2a). Its anatomical position is that of the postoral tentacles, but it lacks their characteristic branches. Hence its anatomical affinities remain obscure.

The animals moved only short distances. Maximum linear distance traversed in three weeks was about 10 cm.

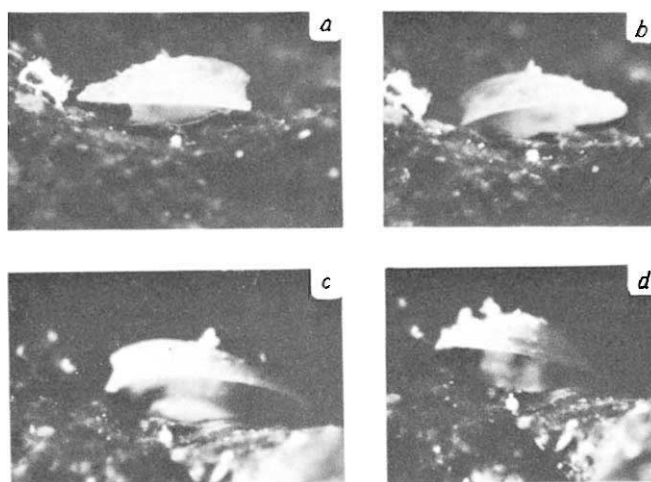


Fig. 2 Various positions of a captive live specimen on a phosphorite nodule. a, Sideview showing partially exposed foot and (?) oral tentacle. Note dark pigmentation on exposed body parts and the distal gill rami below posterior shell margin. b, Posterior view of foot with bulging pedal muscles. c, Lateral view of highly extended foot structure with clearly outlined muscle bulges. The ill defined head (velum) outline is due to motion during photography. d, Posterior view of highly elevated shell above foot. The large dark patch in the proximal foot region, as seen under the microscope and in Kodachrome pictures, is a dark reddish brown. ( $\times 21$  and  $\times 22$ ).

This *Vema* species demonstrates that the Monoplacophora were not restricted to deep sea habitats for all post-mid-Devonian time. This points to the possibility of finding monoplacophoran shells not only in Pleistocene, but even older shallow bathyal deposits.

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H. A. LOWENSTAM

Division of Geological and Planetary Sciences,  
California Institute of Technology,  
Pasadena, California 91125

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## Supernumerary limbs in the axolotl

THE polar coordinate model of French *et al.*<sup>1</sup>, commonly called the clockface model, is a concept of great significance to developmental biology, as it presents a unified view of pattern regulation in *Drosophila* imaginal disks and insect and amphibian limbs. By postulating two rules, intercalation by the shortest route and the complete circle rule for distal transformation, the production of supernumerary limbs in insects<sup>2-5</sup> and amphibians<sup>6,7</sup> after axial reversal is elegantly explained. Following  $180^{\circ}$  rotation, the insect limb can produce two supernumeraries in various positions<sup>2-4</sup>, whereas in the newt the extra limbs are in constant circumferential positions<sup>8</sup>. Because a complete circle is needed for distal transformation, the model clearly predicts that at no other angle of ipsilateral rotation apart

from  $180^\circ$  will supernumeraries be induced. However, Wallace (personal communication), the first to test such a prediction on amphibian limbs, has demonstrated that after  $90^\circ$  or  $270^\circ$  rotation, supernumerary limbs are produced. We report here that, unlike newts and more like insects, axolotls can produce supernumerary limbs anywhere on the circumference after  $180^\circ$  rotation of blastemas. In addition, supernumeraries can be produced at a variety of angles of rotation between  $45^\circ$  and  $315^\circ$ . This work calls for a reappraisal, at least in axolotls, of the clockface model.

The experiments were carried out on young *Ambystoma mexicanum*, 80–140 mm long, whose limbs had been amputated through the mid-humerus level and allowed to regenerate to the late bud–early palette stage<sup>9</sup>. At this point, the blastemas were rotated and, to determine the precise angle of rotation (and to observe any ensuing change), the following marking system was developed. A tungsten needle was dipped into carmine, inserted under the skin proximal to the blastema/stump boundary and pushed into the blastema between the epidermis and the mesenchyme. On withdrawal of the needle a thin red line was apparent, extending from the blastema back into the stump. The blastema was then cut off through the red line, rotated and replaced, any protruding cartilage having been trimmed off. Thus, the now broken line revealed the angle of rotation. As the base of upper arm blastemas is circular in cross-section, the blastema and stump matched perfectly, with no exposed areas. Control grafts were marked with carmine, cut off and replaced. Wound healing occurred in a cold room ( $4^\circ\text{C}$ ) and those cases where irregularities occurred were discarded. The animals were observed twice weekly for several months, during which time the carmine, in most cases, remained visible.

The first series consisted of  $180^\circ$  ipsilateral rotations. Some of the blastemas were marked with carmine, but the majority were exchanged between black and white axolotls so that the grafts were of the opposite colour to the stump. This colour difference permitted the unequivocal identification of the graft and also provided an estimate of the relative graft/host contribution to the supernumeraries. About half of the blastemas de-rotated, but all of those that did not de-rotate produced supernumeraries (Table 1). Twenty-four of the best limbs, where the outgrowths had clearly separated, were analysed for position and handedness (Figs 1 and 2). In Table 1 it can be seen that there is no favoured quadrant for the origin of supernumeraries. This analysis included some that were located exactly at the poles (Figs 1 and 2) and thus it can be concluded that after  $180^\circ$  rotation supernumerary limbs can arise anywhere on the circumference. In those cases where

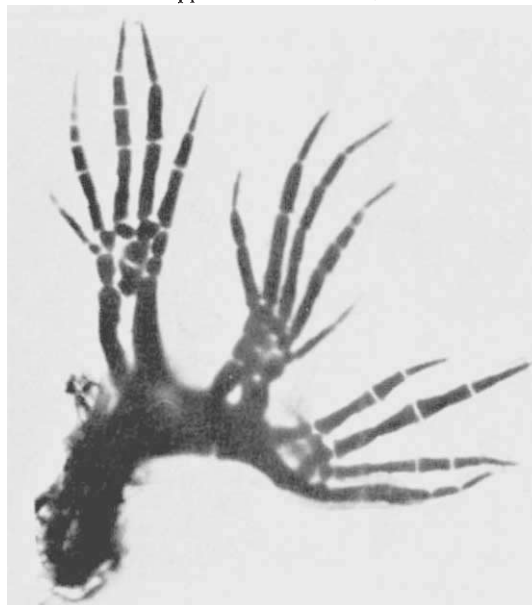


**Fig. 1** The result of rotating a right forelimb blastema through  $180^\circ$ . The limb on the left is a left-handed supernumerary which appeared at the dorsal pole; in the middle is the right-handed rotated blastema; and on the right is a second, partial supernumerary consisting of a humerus, one small zeugopodial element, two carpals and one digit. Note the three-branched humerus and the similarity between this limb and those reduplicated limbs produced in axolotl embryos (Fig. 5A in ref. 13). Victoria blue staining cleared in methyl salicylate.

two supernumeraries arose they were rarely diametrically opposed; instead, the angle between them varied from  $180^\circ$  to  $90^\circ$ . However, in all cases the pairs were of opposite handedness.

A certain proportion (21%) of blastemas also de-rotated after other angles of rotation. By observing the carmine marks it was noted that they always de-rotated by the

**Fig. 2** The result of rotating a right hindlimb blastema through  $180^\circ$ . On the left is a left-handed supernumerary which appeared at the posterior pole; in the middle is the rotated right-handed blastema; and on the right is a right-handed supernumerary which appeared in the anterior-dorsal quadrant. Note that the two supernumeraries are of opposite handedness.



**Table 1** The number and position of supernumeraries after  $180^\circ$  blastemal rotation

| No. analysed | No. forming 1 super | No. forming 2 supers | Position of supernumeraries |            |            |            |
|--------------|---------------------|----------------------|-----------------------------|------------|------------|------------|
|              |                     |                      | AD                          | PD         | PV         | AV         |
| 24           | 11<br>(46%)         | 13<br>(54%)          | 12<br>(32%)                 | 9<br>(24%) | 8<br>(22%) | 8<br>(22%) |

52 blastemas were rotated, of which 29 (56%) de-rotated and 31 (60%) produced supernumerary limbs. Thus, all of those that did not de-rotate resulted in supernumeraries. In addition, some of those that did de-rotate still produced supernumeraries, and in those marked with carmine it was observed that supernumerary outgrowths were induced before axial realignment. Most of these rotations were carried out between black and white axolotls, and from the area of tissue invaded by melanophores an estimate of the relative contribution of host and graft to the supernumeraries could be made. The proportion varied between the limits of 1.3–3:1 graft–host, that is, supernumeraries are composed of both graft and host tissues. AD, anterior–dorsal; PD, posterior–dorsal; PV, posterior–ventral; AV, anterior–ventral.



**Table 2** The number and position of supernumeraries after various angles of ipsilateral rotation

| No. and angle    | No. forming supers | No. of supernumeraries |         |        | Position of supernumeraries |         |         |         |
|------------------|--------------------|------------------------|---------|--------|-----------------------------|---------|---------|---------|
|                  |                    | 1                      | 2       | 3      | AD                          | PD      | PV      | AV      |
| 33 (20°–315°)    | 12 (36%)           | 7 (58%)                | 4 (33%) | 1 (9%) | 6 (35%)                     | 6 (35%) | 3 (18%) | 2 (12%) |
| 22 (0° controls) | 0                  | —                      | —       | —      | —                           | —       | —       | —       |

Blastemas were rotated in an ADPV direction—clockwise for left limbs, anticlockwise for right limbs. 42 limbs were operated upon, 9 (21%) of which de-rotated. The 12 limbs which did result in supernumeraries had been rotated through the following angles: 20°, 45° (2), 75°, 90° (3), 105°, 120°, 135°, 150° and 315°. The angle of rotation was, of course, an estimate, accurate to about 20°. The number, handedness and position of supernumeraries all seemed to vary. Some were of opposite handedness to the stump, some the same, and two were double posterior limbs (Fig. 4). One control limb produced five digits, the other 21 were normal.

shortest route back to 0°. This fascinating phenomenon also occurs in rotated insect legs and epidermal grafts; why some blastemas did not de-rotate at all remains an enigma. In Table 2 the incidence of supernumerary limbs after various rotations is recorded. Again, there seemed to be no favoured segment from which they arose, except perhaps the whole dorsal half, which produced 64% of the limbs. The minimum angle at which some form of outgrowth occurred was 20°, but this was only a two/three digit limb of simple structure. However, at 45°, two limbs produced two supernumeraries each (of the same handedness as the stump), so the minimum angle for the induction of a supernumerary limb might lie between these two values. Up to 180°, the frequency of induction was 50%, then at angles greater than 180° only one more occurred (12%); this was at 315° (Fig. 3). It is doubtful that these frequencies are significant, as Wallace obtained supernumeraries after 270° rotations. It was immediately apparent that the type of limb produced in this series was much more variable than after 180° rotation. Some were of opposite handedness to the stump, some the same, and there were two cases (one at 75° and one at 90°) where double posterior limbs occurred (Fig. 4). The latter outgrowths seem to contradict the complete circle rule which forms the basis of the clock-

face model. Twenty-two control limbs were similarly treated but not rotated (Table 2), one of which produced five digits, the other 21 perfect limbs. This series is currently being repeated on a greater number of animals to determine whether the supernumeraries produced at any one angle are of predictable handedness and/or location.

These results clearly present problems for an interpretation in terms of the clockface model in its present form.



**Fig. 3** The result of rotating a left forelimb blastema through 315°. On the left is the rotated blastema, the fourth digit of which is missing. The limb on the right is a right-handed supernumerary, again without the fourth digit.



**Fig. 4** The result of rotating a left forelimb blastema through 90°. On the left is the rotated blastema, of which the fourth digit is missing and the first digit reduplicated. On the right is a supernumerary limb which can only be described as a double posterior limb.

The 180° rotations can be explained by adopting the original evenly spaced numbering system, as used in the insect leg, which permits supernumeraries to occur anywhere. Such an organisation predicts that pairs of supernumeraries will be of opposite handedness, as indeed they were. Yet this cannot then explain the supernumeraries produced by other angles of rotation. On the other hand, the reallocation of numbers needed to permit maximum incongruity after a rotation such as 45° then becomes incapable of explaining the other experimental results. It might be necessary, therefore, to reject the complete circle rule, particularly in view of the production of double posterior limbs (Fig. 4). The results of other work with axolotls, such as the regeneration of double half hind limbs (D. L. Stocum, unpublished data) and the regeneration of embryonically produced duplicated limbs<sup>10</sup>, also lead to the

same conclusion. Yet one still has to find an explanation for the failure of double half forelimbs to regenerate (ref. 11, S. V. Bryant, B. A. Baca and P. W. Tank, unpublished data). It is conceivable that these paradoxes might be resolved by the consideration of a double gradient system<sup>12</sup>, where one gradient corresponds to the antero-posterior axis, the other to the dorso-ventral axis. Such a system provides a simple physiological mechanism for the postulated circumferential gradient of the clockface model, and conforms to the classical embryological work concerning the individual determination of limb bud axes. The ability of a double gradient to explain the above results is currently being investigated.

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M. MADEN\*

Division of Developmental Biology,  
National Institute for Medical Research,  
Mill Hill, London NW7, UK

R. N. TURNER

Developmental Biology Group,  
School of Biological Sciences,  
University of Sussex, Brighton, Sussex, UK

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\*To whom correspondence should be addressed.

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## Mycolase, a new kind of systemic antimycotic

THERE is a shortage of antimycotic drugs<sup>1,2</sup>, especially those for the treatment of systemic fungal diseases. Patients on immunosuppressive therapy or cancer chemotherapy are particularly susceptible to opportunist fungal infections<sup>3</sup>, and histoplasmosis, blastomycosis and coccidioidomycosis are major health problems in some tropical countries<sup>4</sup>. For such infections, amphotericin B, 5-fluorocytosine, micazone and clotrimazole are mainly used. The first of these drugs is nephrotoxic and the second often induces resistant strains of fungi<sup>5</sup>. Griseofulvin is given orally but is effective only for cutaneous infections<sup>6</sup>. A new approach, which we are investigating, is to attack the fungal cell wall by way of the glucans it contains. Preliminary results obtained with 'mycolases', mixtures of enzymes of fungal origin containing various carbohydrases, including chitinase and laminarinase are reported here. We found that, used in conjunction with normally ineffective antimycotic drugs, mycolases were successful against systemic fungal disease in mice.

Most true fungi contain chitin in their cell wall<sup>7,8</sup>; it probably always occurs in the innermost layer but may also be present in outer layers of the surface material as one of various polysaccharides which protect these and other microorganisms<sup>9,10</sup>. Although the pattern and general cell-wall architecture varies among different species, chitin has special importance, as shown by the inability of fungal protoplasts to revert to a viable mycelium without a source of the chitin precursor, N-acetyl-D-glucosamine<sup>11</sup>.

Chitin has been recognised by other workers as a

potential target for therapeutic attack, and specific inhibitors of chitin synthetase have been developed. These compounds, the polyoxins, are unstable *in vivo* and have not been useful clinically<sup>12</sup>. Chitinases occur widely in nature, and although it has been suggested that chitinase might be used to attack and kill chitin-containing microbial pathogens<sup>13</sup>, no such effect has been recorded, either *in vitro* or *in vivo*. Because chitin does not occur in any part of the mammalian body structure, chitinase might be expected to be nontoxic to mammals. We have found this to be the case, but chitinase alone is not an effective mycostatic or mycoid agent (see below).

We have investigated the effect of certain carbohydrases on fungal mycelium by measuring the extent of protoplast formation<sup>14</sup>. Although assays of the enzymatic degradation of isolated mycelial wall components of target pathogens provide useful estimates of activity *in vitro*, the structure and composition of mycelia vary widely between species and are insufficiently understood to relate enzyme levels to *in vivo* activity.

**Table 1** Production of protoplasts from vegetative mycelium of *Aspergillus fumigatus*

| Enzyme treatment         | Protoplast release |
|--------------------------|--------------------|
| Chitinase*               | +                  |
| Laminarinase†            | —                  |
| Chitinase + laminarinase | ++                 |
| <i>Coprinus</i> extract‡ | +++                |

All enzymes were at a final concentration of 240 µg ml<sup>-1</sup>.

\*β-1, 4-N-acetyl-D-glucosaminidase (EC3.2.1.14, crystalline, Koch-Light).

†β-1,3(4)-glucanase (EC 3.2.1.6, BDH).

‡ Extract of *Coprinus comatus* (shaggy ink cap), centrifuged, dialysed and lyophilised (mycolase I).

§+, Protoplast release from hyphal tips only; ++, extensive formation of protoplasts; +++, complete lysis of mycelium; —, no protoplasts produced.

Table 1 indicates that chitinase alone has no access to its substrate in *Aspergillus*, except near growing tips of the mycelium, where chitinase and chitin synthetase coexist in a state of physiological collaboration to extend the mycelium in normal growth<sup>15</sup>. Laminarinase provides access for the chitinase by degrading surface glucans, so forming an effective combination with chitinase, but alone it is not mycolytic. Natural enzyme mixtures, for example, mycolase I (from *Coprinus*), are much more effective because of the presence of a wider range of carbohydrases. Similar results can be obtained with *Candida albicans* and many other microfungi as target organisms. Enzyme profiles for mycolases will be reported elsewhere.

*In vivo* assays led to similar conclusions and were carried out by infecting mice by intravenous injection, followed by various treatments with enzymes and/or conventional antimycotic drugs. Results shown in Table 2 are from tests in which BALB/c mice given  $5 \times 10^6$  spores of *Aspergillus fumigatus* died, if untreated, in 12-14 d. Groups of such infected mice were given intraperitoneal injections of various enzyme preparations, and results are shown for mycolase I (from *Coprinus comatus* fruit bodies) and mycolase III (from *Lycoperdon pyriforme* grown in culture). These were tested with and without amphotericin B or nystatin.

Our conclusions are drawn only from comparisons between groups of mice in which all animals died and other groups in which all survived. In the conditions of the test, maximal sub-toxic doses of the drugs failed to prevent death from fungal infection (Table 2 *i, k*). Animals could be saved by enzyme mixtures alone (*d, h*) or by the synergistic effect of smaller doses of enzyme and drug (for example, *m, n, o, p*). When mice were infected with *A. fumigatus*



after immunosuppression with rabbit anti-mouse thymocyte globulin (ATG), they could still be treated effectively with enzyme plus drug, indicating that a full immune capacity is not a prerequisite for the effect (*q, r*).

Protoplasts of *C. albicans* are more sensitive to amphotericin B than intact cells<sup>17</sup>, and we expected that enzymatic damage of the fungal cell walls would facilitate penetration by the existing antimycotic drugs. Although this is certainly true, we have been surprised by the ability of mycolases alone to cure fungal infections.

Acute toxicity tests with mycolases, given intravenously or intraperitoneally, gave an LD<sub>50</sub> of 15–20 mg per mouse. These data gave a favourable therapeutic index of 150–200. Enzyme preparations fractionated by ethanol precipitation were non-pyrogenic and showed no short-term or longer-term toxic effects in monkeys given a single intravenous injection of 10 mg per kg. Clinical experience will be presented elsewhere.

Mycolases are only poorly immunogenic, and if these enzyme mixtures were used to treat systemic infections in transplant patients or patients receiving cancer chemotherapy, the risks of sensitisation would be minimised by immunosuppression and impaired immunocompetence, respectively. Superficial infection, such as ringworm (*Trichophyton mentagrophytes*) on guinea pig skin, can be

treated effectively by topical application of mycolase plus drug; in this kind of situation sensitisation is unlikely to be a problem.

Systemic fungal disease is notoriously difficult to diagnose (most often identified after death) and can take a very rapid course once established. There is a need for effective nontoxic antimycotic agents and we believe that mycolases provide an acceptable treatment for systemic fungal disease.

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D. A. L. DAVIES

A. M. S. POPE

Department of Immunology,  
Searle Research Laboratories,  
Lane End Road, High Wycombe, Bucks, UK

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**Table 2** Fate of BALB/c mice infected systemically with *Aspergillus fumigatus*

| Treatment                                                | Injections<br>(d after<br>challenge) | Dose                       | Mean survival*<br>(d) |
|----------------------------------------------------------|--------------------------------------|----------------------------|-----------------------|
| a None (controls)                                        | —                                    | —                          | 13.5                  |
| b Chitinase + laminarinase                               | 1,2,3                                | 3 × 1.5 mg                 | 26.3                  |
| c <i>Coprinus</i> (crude)                                | 1                                    | 1.5 mg                     | 21.0                  |
| d <i>Coprinus</i> (crude)                                | 1,2,3                                | 3 × 1.5 mg                 | All survived*         |
| e <i>Coprinus</i> (crude)                                | 12                                   | 4.5 mg                     | 35.6                  |
| f <i>Lycoperdon</i> (crude)                              | 1                                    | 1.5 mg                     | 11.2                  |
| g <i>Lycoperdon</i> (purified)†                          | 1                                    | 100 µg                     | 15.8                  |
| h <i>Lycoperdon</i> (purified)                           | 1,2,3                                | 3 × 100 µg                 | All survived*         |
| i Amphotericin B                                         | 1                                    | 5.0 µg                     | 23.2                  |
| j Amphotericin B                                         | 1                                    | 0.6 µg                     | 16.0                  |
| k Nystatin                                               | 1                                    | 100 µg                     | 30.6                  |
| l Nystatin                                               | 1                                    | 12.5 µg                    | 14.6                  |
| m <i>Coprinus</i> plus amphotericin (c + j)              | 1–2                                  | 1.5 mg + 0.6 µg            | All survived*         |
| n <i>Coprinus</i> plus nystatin (c + l)                  | 1–2                                  | 1.5 mg + 12.5 µg           | All survived*         |
| o <i>Lycoperdon</i> plus amphotericin (f + j)            | 1                                    | 1.5 mg + 0.6 µg            | All survived¶         |
| p <i>Lycoperdon</i> (purified) plus amphotericin (g + j) | 1–2                                  | 100 µg + 0.6 µg            | All survived¶         |
| q ATG before challenge‡                                  | –1                                   | 300 µl                     | 8.6                   |
| r ATG (q), plus <i>Coprinus</i> plus amphotericin (m)    | –1 (ATG) 2<br>1–4                    | 2 × 1.5 mg–<br>2 × 0.6 µg§ | All survived*         |

Groups of mice, usually 5 but in some cases 10, weighing between 22 and 25 g, were challenged by intravenous injection with  $5 \times 10^6$  spores of *A. fumigatus*. Control (untreated) mice usually died after 12–14 d (a); the time was 8.6 d for control mice in the case of h. Enzymes and drugs were used as described in Table 1. The toxic doses of amphotericin B and nystatin were approximately 10 µg and 200 µg, respectively. Treatment was administered intraperitoneally.

\* Significantly different from a at 1% level.

† *Lycoperdon* (*L. pyriforme*) was grown in culture and the culture supernatant fluid used untreated ('crude') or fractionated by gel filtration (the retarded fraction of Sephadex G200 columns was used), and referred to as 'purified'.

‡ ATG is rabbit anti-mouse thymocyte globulin; this was injected subcutaneously and the amount used (300 µl) was that able to prolong the survival of allogeneic skin grafts from 8–10 d to 25–35 d.

§ In this test ATG was injected the day before challenge at the same dose as in q. Mycolase I was given on days 1 and 3 (dose 1.5 mg) and drug on days 2 and 4 (dose 0.6 µg) after challenge.

¶ Significantly different from a, f and j at 1% level (Log rank  $\chi^2$  analysis<sup>16</sup>).

## Inherited disorders in the regulation of serum calcium in rats raised from parathyroidectomised mothers

In a series of experiments to study the influence of maternal endocrine dysfunction on the embryonic development in rats, we have found that the first generation (F<sub>1</sub>) rats born to parathyroidectomised (Px) mothers possess a slightly, but significantly, lower serum calcium level than in normal control rats<sup>1</sup>. Furthermore, the serum calcium level in the rats of the third or fourth generations (F<sub>3</sub> or F<sub>4</sub>) developed by brother–sister mating was still at a subnormal level. To characterise calcium metabolism in these animals, the response of serum calcium to removal of the parathyroid gland was examined in the F<sub>1</sub>, F<sub>3</sub> and F<sub>4</sub> rats. We report here that F<sub>3</sub> and F<sub>4</sub> rats raised from mothers parathyroidectomised on the 5th day of pregnancy showed a less marked decline in serum calcium level following parathyroidectomy as shown in the first generation, suggesting that the functional alteration resulted from the intrauterine hypocalcaemia persists in subsequent generations.

Female rats of highly inbred Wistar–Imamichi strain, weighing 250–300 g, were obtained from the Animal Breeding Research Laboratory (Ohmiya) and were mated. The day on which sperm were present in the vaginal smears was taken as day 0 of pregnancy. The surgical parathyroidectomy was carried out on the 5th day of pregnancy under ether anaesthesia. After the operation, mother rats were given 1% calcium chloride solution as drinking water for 3 d before parturition and for the first 3 d of lactation. Without calcium chloride solution supplementation, they died of tetanic convulsions on the 21st or 22nd day of pregnancy. Control pregnant rats were sham operated. Adult female rats at the F<sub>1</sub> and at the F<sub>3</sub> or F<sub>4</sub>, weighing 250–280 g, were parathyroidectomised unilaterally or bilaterally under ether anaesthesia. All animals used were maintained in an air-conditioned room (22 ± 2 °C, 55 ± 5%) with a lighting schedule of 14 h light (0600–2000) and 10 h

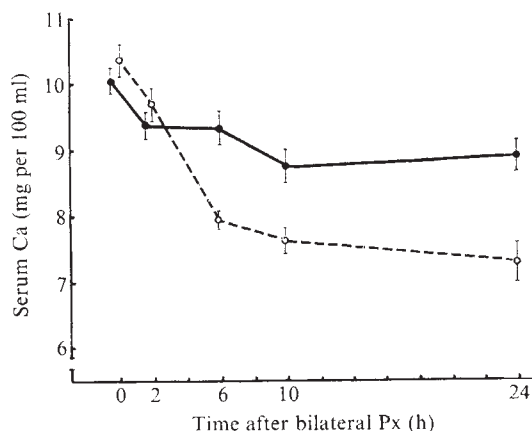
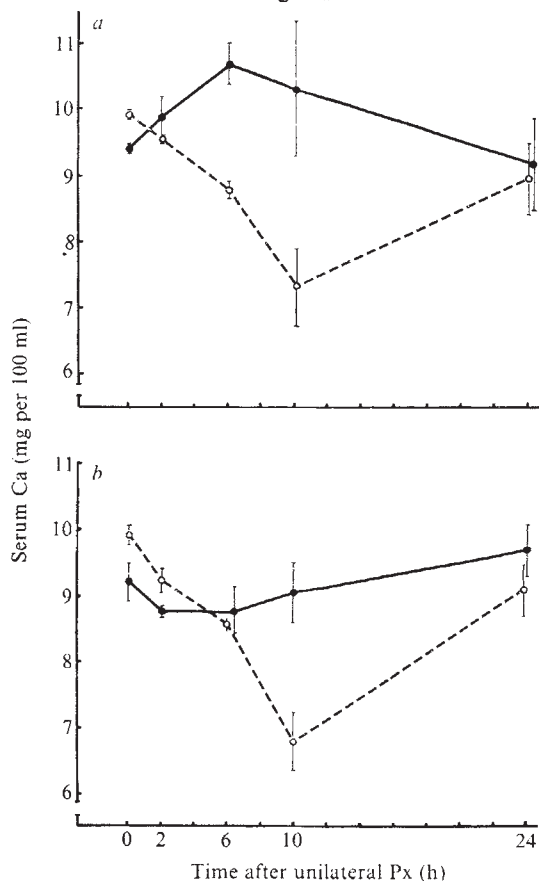
darkness, and were supplied with a commercial stock diet (calcium, 1.01%; phosphorus, 1.17%) and water *ad libitum*. Serum calcium concentrations were determined using *o*-cresolphthalein complexone<sup>2</sup>.

A rapid decrease in the serum calcium concentration occurred when parathyroid gland was removed unilaterally in normal control rats (Fig. 1*a, b*); calcium levels began to decrease at 2 h and reached a minimum value of  $7.33 \pm 0.51$  mg per 100 ml serum (Fig. 1*a*) or  $6.75 \pm 0.36$  mg per 100 ml serum (Fig. 1*b*) 10 h after the operation. They returned towards the subnormal level at 24 h. In contrast, serum calcium in the  $F_1$  rats increased slightly 6 and 10 h after the operation and then decreased below the initial level at 24 h (Fig. 1*a*).  $F_3$  rats did not show any significant decrease in serum calcium levels during a 24-h period after unilateral parathyroidectomy (Fig. 1*b*) and in further detailed study, it did not show any further sharp decline when examined 13, 48, 72 h and 1 week after operation (to be published elsewhere). Initial calcium levels in the rats of the  $F_1$  and  $F_3$  groups were slightly lower than those in normal controls.

Bilateral parathyroidectomy was followed by a marked fall in the serum calcium within 6 h in normal control rats (Fig. 2). In the  $F_4$  rats, the decrease in calcium concentration was less marked for the first 24 h after bilateral parathyroidectomy (Fig. 2).

Blood calcium concentration is regulated by the two antagonistic calcium-regulating hormones<sup>3-6</sup>, parathyroid hormone (PTH) and calcitonin (thyrocalcitonin), and by the metabolites of vitamin D (refs 7 and 8). The prompt

**Fig. 1** Time course of the effect of unilateral surgical removal of the parathyroid gland (unilateral Px). *a*, On serum calcium concentrations in adult female rats of normal control group (---) and of the first generation born to a mother parathyroidectomised on the 5th day of pregnancy (—). Each value represents the mean  $\pm$  s.e.m. of five animals; *b*, On serum calcium concentrations in adult female rats of normal control group (---) and of the third generation raised from parathyroidectomised mothers (—). Each value represents the mean  $\pm$  s.e.m. of eight animals.



**Fig. 2** Time course of the effect of bilateral surgical removal of parathyroid glands (bilateral Px) on the serum calcium concentrations in adult female rats of normal control group (---) and of the fourth generation raised from parathyroidectomised mothers (—). Each value represents the mean  $\pm$  s.e.m. of five or six animals.

and transient fall in the systemic blood calcium following unilateral parathyroidectomy in normal rats might suggest that the activity of thyrocalcitonin became predominant in an acute phase of hypocalcaemia induced by parathyroidectomy, because of a rapid reduction in the amount of circulating PTH. Half life of PTH has been reported to be approximately 20 min (ref. 9). In addition, thyrocalcitonin released from the thyroid gland after surgical parathyroidectomy<sup>6</sup> could be another contributory factor to the manifestation of the acute hypocalcaemia. If this view is correct, the decreased responsiveness to unilateral parathyroidectomy in the  $F_1$  and  $F_3$  rats of the Px group may be due to a decreased synthesis and/or release of thyrocalcitonin in these animals.

The prolonged decline in the serum calcium level after bilateral parathyroidectomy has generally been considered to be the result of removal of the PTH source and of the secondary manifestations of the withdrawal of the effect of the hormone on the bone, kidney and gut. It is well established that PTH and calcitonin regulate bone resorption<sup>10,11</sup>, renal tubular reabsorption of calcium<sup>12,13</sup> and calcium absorption from the gut<sup>14</sup>. The results obtained in the  $F_4$  rats following bilateral parathyroidectomy may suggest that an altered responsiveness of these organs to the calcium-regulating hormones could be an additional factor in the maintenance of the relatively normal level of serum calcium after parathyroidectomy in the rats raised from parathyroidectomised mothers.

The present results show clearly that a characteristic response of serum calcium to the deprivation of PTH, which was shown in the  $F_1$  rats born to a mother parathyroidectomised on the 5th day of pregnancy, was inherited into the  $F_3$  and  $F_4$  generations. These observations suggest that resistance to acute hypocalcaemia, as a consequence of the removal of the parathyroid gland, must be acquired during embryonic development within a hypocalcaemic mother rat.

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T. FUJII\*

Department of Pharmacology,  
Tokyo Women's Medical College,  
Tokyo 162, Japan

Received 10 January; accepted 16 March 1978.

\*Present address: Department of Pharmacology, Teikyo University School of Medicine, Tokyo 173, Japan.

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## Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus

THERE is remarkably little information about the possible importance of cell-mediated immune responses in the protection of hosts during influenza virus infection. It has recently been found that specific cytotoxic T cells ( $T_c$ ) can be recovered from the spleens of mice previously inoculated intranasally or intravenously with live virus<sup>1–4</sup> and peak activity occurs about 6 d after virus inoculation. Furthermore,  $T_c$  are found in the lungs and the lymph nodes draining the lungs of infected mice in conditions which suggest that these cells might be important in host recovery from this infection<sup>5</sup>. Adoptive transfer of primary or secondary immune spleen cells to mice inoculated intranasally with a lethal dose of A/WSN virus caused a significant reduction of infectious virus levels in the lungs and prevented death<sup>6</sup>. The active cells in the transferred population were T lymphocytes. This report shows that the protective effects conferred by the transferred T cells in these conditions are largely, if not entirely, due to  $T_c$ .

Procedures for inoculation (intravenous and intranasal) of A/WSN influenza virus into CBA/H mice, the preparation of primary (*in vivo*) and secondary (*in vitro*) effector T cells, assays for cytotoxic activity on infected, syngeneic target cells<sup>1,5,6</sup>, and the preparation, properties and conditions of use of anti-Ly 1.1 and 2.1 sera<sup>7–9</sup> for the lysis of  $T_c$  and helper T cells respectively were as described previously. For the H-2 restriction experiment, A.TL and A.TH mice were inoculated with virus in the same conditions as CBA mice. The H-2 haplotypes of these mice are given in Table 1.

There are three classes of T lymphocytes which could contribute to the immune response of the host to viral infection. These are helper T cells ( $T_h$ ), T cells taking part in delayed type hypersensitivity ( $T_d$ ) and  $T_c$ . Little is known about the generation of  $T_d$  or  $T_h$  in the conditions of these experiments, but Fig. 1 shows that there is a striking correlation between the cytotoxic activity of injected

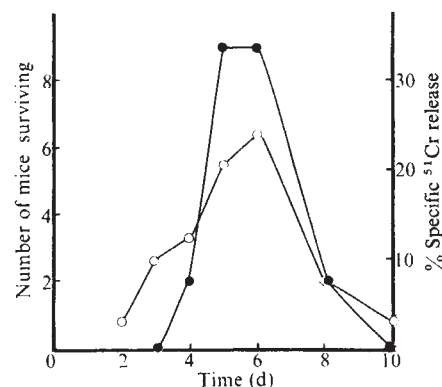


Fig. 1 CBA mice were injected intravenously with  $10^7$  EID<sub>50</sub> A/WSN virus at 2, 3, 4, 5, 6, 8 and 10 d before removal of their spleens. T cell-mediated cytotoxicity assays were carried out, and the results shown (○) against time (d) when spleens were removed after intravenous injection of virus.  $1 \times 10^6$  cells from each preparation were injected intravenously into each mouse in groups of nine mice 24 h after intranasal inoculation of a lethal dose ( $10^3$  EID<sub>50</sub>) of A/WSN virus and the survival of the mice over a period of 21 d noted (●).

primary immune spleen cells and their capacity to protect mice from death after intranasal inoculation of a lethal dose of virus. Spleen preparations with the highest cytotoxic activity gave complete protection against death. It is possible to distinguish between  $T_c$  compared with  $T_d$  and  $T_h$  in two ways. T cell-mediated lysis of influenza virus-infected target cells requires H-2K or H-2D region homology between the donors of cytotoxic T cells and infected target cells<sup>1</sup>. In contrast, interaction of  $T_h$  and  $T_d$  cells with their appropriate cellular partners requires homology in the I region<sup>10–13</sup>. A protective role played by these subsets could be determined therefore by adoptive transfer of effector cell populations into hosts which were similar or different at the K, D or I regions of the H-2 complex<sup>14,15</sup>. The results of such an experiment using secondary immune spleen cells is shown in Table 1. Transfer of such cells into hosts which were syngeneic or were homologous at the K and D regions, but differed in the I region, reduced infectious virus titres in the lungs by 2–4 log, in each case a significant reduction. Transfer of cells into a host (CBA/H) with I region, but not K and D region homology, failed to cause a reduction in the lung viral titres. The results are consistent with the interpretation that  $T_c$ , and not  $T_h$  or  $T_d$ , play a major part in reducing lung virus titres. It could be argued that the lack of effect of the transferred A.TL cells into CBA/H hosts was due to a host against graft reaction, although it seems unlikely this would have been so pronounced as to cause complete rejection of the injected cells within a few days of transfer.

Table 1 H-2 requirement for transfer of protective activity\* with splenic immune T cells†

| Immune cells transferred‡<br>Strain | H-2 haplotype | Strain | Virus titre in recipients' lungs§<br>H-2 haplotype | Titre             | Virus titre compared to control groups given no cells |
|-------------------------------------|---------------|--------|----------------------------------------------------|-------------------|-------------------------------------------------------|
| —                                   | —             | A.TL   | skkkkd                                             | $6.74 \pm 0.14$   |                                                       |
| A.TL                                | skkkkd        | A.TL   | skkkkd                                             | $4.16 \pm 1.90$ ¶ | –2.48                                                 |
| —                                   | —             | A.TH   | sssssd                                             | $6.55 \pm 0.20$   |                                                       |
| A.TL                                | skkkkd        | A.TH   | sssssd                                             | $3.58 \pm 1.00$ ¶ | –2.97                                                 |
| —                                   | —             | CBA/H  | kkkkkk                                             | $6.22 \pm 0.51$   |                                                       |
| A.TL                                | skkkkd        | CBA/H  | kkkkkk                                             | $6.21 \pm 0.31$   | +0.01                                                 |

\*Expressed as the reduction in virus level in the lungs of recipients.

†Raised *in vitro* by restimulation of spleen cells from mice primed with the same virus (A/WSN) 21 d or more previously.

‡ $2 \times 10^7$  cells transferred to each recipient 24 h after virus instillation ( $0.5 \times 10^3$  EID<sub>50</sub> per recipient).

§Expressed as mean log<sub>10</sub> EID<sub>50</sub> per lung extract  $\pm$  s.d. of the mean in groups of four mice 5 d after cell transfer.

¶Significantly different from control mice given no cells ( $P < 0.05$ ).

||Not significantly different from control mice given no cells ( $P > 0.80$ ). Transfer of  $2 \times 10^7$  CBA immune cells to CBA mice caused an average reduction in lung virus titres of 3.3 log<sub>10</sub> (ref. 6).

**Table 2** Effect of anti-Ly and complement treatment on protective activity\* of splenic cytotoxic T cells†

| Treatment                     | Virus titre in recipient lungs§ | Virus titre compared with control group |
|-------------------------------|---------------------------------|-----------------------------------------|
| Untreated                     | 0.43±1.8                        | -4.70                                   |
| α-Ly 1.1+C'                   | 0.77±0.8¶                       | -4.36                                   |
| α-Ly 2.1+C'                   | 5.45±0.4**                      | +0.32                                   |
| Control group, no cells given | 5.13±0.1                        |                                         |

\*Expressed as a reduction in virus level in the lungs of recipients.

†Raised *in vitro* by restimulation of spleen cells from mice primed with the same virus (A/WSN) 21 d previously.

‡ $3 \times 10^7$  cells were resuspended in 0.2 ml medium plus 0.1 ml α-Ly serum at room temperature for 30 min. The washed cells were resuspended in 1 ml normal rabbit serum (absorbed twice with normal BALB/c and CBA/H spleen cells) at 37 °C for 25 min; washed twice and the number of viable cells counted.  $2.5 \times 10^7$  untreated cells, or viable cells prepared after α-Ly treatment were injected into each recipient 24 h after virus instillation ( $0.5 \times 10^3$  EID<sub>50</sub>).

§Expressed as mean log<sub>10</sub> EID<sub>50</sub> per lung extract ± s.d. of the mean in groups of three mice 5 d after cell transfer.

||Significantly different from control mice given no cells ( $P < 0.01$ ).

¶Significantly different from control mice given no cells ( $P < 0.001$ ) α-Ly 1.1+C' treatment lysed 38% of the cells and reduced cytotoxic activity by 29%.

\*\*Not significantly different from control mice given no cells. ( $P > 0.4$ ). α-Ly 2.1+C' treatment lysed 60% of the cells and reduced cytotoxic activity by 66%.

For this reason additional evidence was sought to confirm the role of T<sub>c</sub>. It has been shown in several systems that T<sub>c</sub> compared with T<sub>h</sub> and T<sub>d</sub> have different antigen markers on their surfaces, so that they may be distinguished by appropriate antisera; T<sub>h</sub> and T<sub>d</sub> react with anti-Ly 1.1 serum and T<sub>c</sub> with anti-Ly 2.1 serum. Secondary (CBA/H) effector cells were therefore treated with anti-Ly 1.1 or anti-Ly 2.1 serum and complement, or were untreated. The remaining cells were then injected into CBA/H mice infected intranasally with  $0.5 \times 10^3$  EID<sub>50</sub> A/WSN virus 24 h previously. Lung virus titres were determined 6 d after virus inoculation. The results (Table 2) show very clearly that treatment with anti-Ly 2.1 serum plus complement, but not with anti-Ly 1.1 serum and complement, destroyed the ability of the cells to reduce the lung virus titres. These findings therefore complement the experiment described in Table 1 and indicate clearly that cytotoxic T cells are able to reduce influenza titres in mouse lungs.

This we believe is the first time a specific subset of immune T cells has been shown to have such an effect in hosts with a respiratory virus infection. The results indicate that T<sub>c</sub> formation is a defence mechanism of the host which is provoked rapidly in response to infection and which occurs before there is production of significant levels of antibody. These cells may also be important in circumstances when antibody at the mucosal surface may not prevent infection of respiratory epithelium.

K. L. YAP  
G. L. ADA

Department of Microbiology,  
John Curtin School of Medical Research,  
Australian National University,  
Canberra, Australia

I. F. C. MCKENZIE

Department of Medicine,  
Austin Hospital,  
Heidelberg, Victoria, Australia

Received 12 December 1977; accepted 30 March 1978.

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## Inverse regulation of cell-mediated and humoral immune responses to oxazolone by H-2 linked genes

THE fate of histoincompatible transplants or malignant cells bearing new antigens on the cell surface may be assumed to depend on the magnitude and proportion of the cell-mediated and humoral immune responses directed towards these new antigens in the host<sup>1-6</sup>. Although the genetic control of both the humoral and the cell-mediated immune responses has been studied intensively<sup>7-12</sup>, far less is known about the genetic regulation determining the magnitude and proportion of these two types of responses to the same antigenic determinant. We report here results of such studies using a simple chemical, oxazolone, which elicits both delayed type hypersensitivity (DTH) and antibody responses.

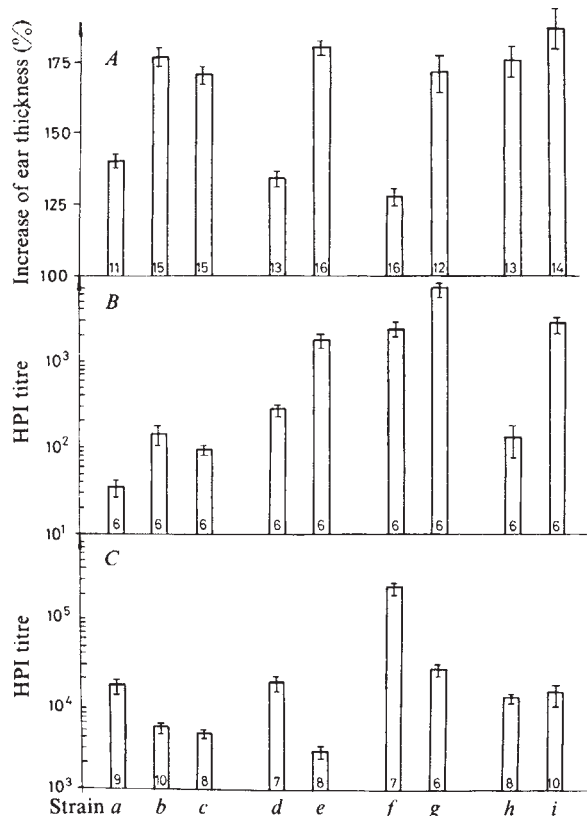
Inbred and H-2 congenic mouse strains were used for the analysis of the genetic factors controlling cell-mediated and humoral immune responses. The results demonstrate remarkable differences in the magnitude of all three responses (Fig. 1a,b,c) between the strains tested. Greater DTH was recorded in strains B10.BR, A/Ph and C3H/Di than in their congenic partners, B10, A.BY and C3H.SW, respectively ( $P < 0.001$ ) (Fig. 1a). According to the magnitude of the anti-oxazolone IgG antibody titre raised by sensitisation with oxazolone (Fig. 1b), the strain distribution pattern of the responsiveness parallels the expression of DTH after sensitisation (Fig. 1a). Contrary to the previous results, if mice of the same strain were immunised with oxazolone conjugated to another protein, mouse serum albumin (Ox<sub>7</sub>MSA), the titre of the anti-oxazolone IgG antibodies was higher in B10, A.BY and C3H.SW, than in their congenic partners, B10.A, B10.BR, A/Ph and C3H/Di mice. These findings indicate a strong association of high and low antibody responsiveness with H-2<sup>b</sup> and H-2<sup>a,k</sup> haplotypes, respectively (Fig. 1c).

Because both the DTH and IgG responses to oxazolone were as high in (B10×B10.A)F<sub>1</sub> and (A/Ph×A.BY)F<sub>1</sub> hybrids as in the high responder parental strains, it can be inferred that the inheritance of high responsiveness is a dominant trait (last two columns of Fig. 1a,b,c). Irrespective of findings that the route and mode of administration of an antigen can have a considerable effect on the type and magnitude of immune responses<sup>2,8,10</sup>, it can be seen that both cellular (DTH) and humoral (IgG) responses to oxazolone are under the control of H-2 genes.

It can be supposed that the same hapten (Ox) and carrier (skin self-protein) conjugate is the trigger for both DTH and IgG responses after sensitisation; therefore, the expression of DTH parallels the magnitude of IgG responses (Fig. 1a,b).

The inverse relationship between the magnitude of the DTH and IgG responses after sensitisation (Fig. 1a,b) and IgG response after immunisation (Fig. 1c) cannot be easily explained, but might be interpreted as a consequence of the presentation of the haptenic determinant on different carriers, and/or the differing mode and route of antigen presentation. It might be supposed, even without direct





**Fig. 1** Cellular and humoral immune responses to oxazolone. *a*, DTH; *b*, secondary IgG response; *c*, IgG response following immunisation with an oxazolone conjugate. Mice were sensitised with 3% oxazolone in ethanol<sup>13,14</sup>. For the induction of DTH, 50  $\mu$ l was used, and for that of secondary IgG response, 2  $\times$  50  $\mu$ l. The DTH to oxazolone was tested 10–11 d after sensitisation<sup>14,15</sup> and expressed as the % increase of the starting ear-thickness at the time of peak response (24 h). MSA was recrystallised 6  $\times$  (ref. 17), passed through a Sephadex G-200 column and coupled to dioxane-dissolver oxazolone. 200  $\mu$ g Ox<sub>17</sub>MSA was injected in complete Freund's adjuvant subcutaneously, and 3 weeks later a further 100  $\mu$ g in phosphate-buffered saline was injected intraperitoneally. All mice were bled 8 d after the secondary stimulus. After 2-mercaptoethanol treatment of the sera, IgG antibody titres were determined by haptenated bacteriophage inactivation assay<sup>18</sup>. The titre was considered as the dilution of the antisera which reduced the number of plaques by 50%. Each column-height represents the arithmetic mean ( $\bar{x}$ ) of the individual values and the bar shows the standard error of the mean ( $S\bar{x}$ ). The number within the column in brackets indicates the number of determinations. *a*, B10; *b*, B10.A; *c*, B10.BR; *d*, A.BY; *e*, A/Ph; *f*, C3H.SW; *g*, C3H/Di; *h*, (B10  $\times$  B10.A) $F_1$ ; *i*, (A/Ph  $\times$  A.BY) $F_1$ .

evidence, that differences in the mode and route of presentation, as well as in the carriers of a haptenic antigen, can result in differences in the induction of T helper and T suppressor cell subpopulations.

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*Note added in proof:* Recently, the mapping of *H-2* genes regulating both responses to oxazolone has been published<sup>19,20</sup>.

J. FACHET  
I. ANDO

Institute of Genetics,  
Biological Research Center,  
Hungarian Academy of Science,  
6701-H Szeged, Hungary

Received 21 December 1976; accepted 20 March 1978.

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## Chronic guanethidine treatment increases cardiac $\beta$ -adrenergic receptors

VARIOUS pharmacologically active agonists capable of altering cell function have been shown to interact primarily with specific receptors on the outer cell membrane<sup>1</sup>. Evidence has accumulated from several systems to suggest that there may be a dynamic and inverse relationship between the number or density of receptors on the membrane surface and the degree of receptor occupancy by agonist<sup>2,3</sup>. We present here experiments which demonstrate in rat hearts that chronic withdrawal of adrenergic stimulation produced by guanethidine treatment *in vivo* results in increased cardiac  $\beta$ -adrenergic receptor density. Further, in isolated, perfused hearts obtained from guanethidine-treated animals, there is an increased cyclic AMP (cAMP) accumulation in response to the  $\beta$ -adrenergic agonist isoprenaline, suggesting that the increased numbers of receptors may be related to the increased biochemical response.

Male C-D strain rats (Charles River) weighing 180–200 g were used in all experiments. The animals were housed in pairs, provided *ad libitum* with food (Purina rat chow) and water, and maintained on a 12-h, 0600–1800 h, alternating light–dark cycle. Animals were killed between 0900 h and 1200 h.

The animals were treated with chronic, high-dose guanethidine according to the method of Johnson and O'Brien<sup>4</sup>. Animals were treated for five consecutive days a week for five weeks with intraperitoneal injections of guanethidine, 40 mg per kg. The drug was made up as a 5 mg ml<sup>-1</sup> solution in isotonic saline adjusted to pH 7.4 before injection. After treatment a 'washout' period of four weeks was allowed before experimentation. Control animals were injected with equal volumes of isotonic saline.

Cardiac  $\beta$ -adrenergic receptor number was assayed in ventricular tissue according to Alexander, with minor modifications<sup>5</sup>. For these experiments 16 control and 16 experimental animals were used. Four groups of four control and four treated animals were killed by cervical dislocation and the hearts rapidly excised. The atria were removed and the ventricles minced with scissors to a particle size of 1–3 mm in 20 ml of ice-cold sucrose (0.25 M), Tris-HCl (5 mM), pH 7.4, and MgCl<sub>2</sub> (1 mM). The ventricular pieces were allowed to settle and the supernatant fluid decanted and replaced with 30 ml of fresh, ice-cold sucrose buffer. The suspension was homogenised at 0 °C using 20 strokes of a motor-driven grooved Teflon-glass homogeniser.

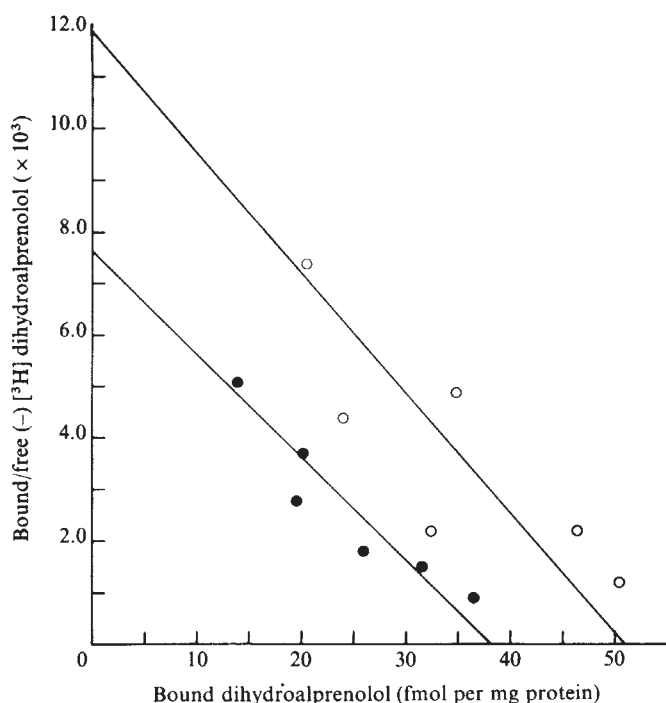
This homogenate (5 ml) was mixed with 0.8 N HClO<sub>4</sub> (5 ml) and centrifuged at 4 °C for 10 min at 30,000g. The supernatant fluid was used for determination of noradrenaline content by fluorimetry<sup>6</sup>. The remainder of the homogenate was filtered through cheese cloth and the filtrate centrifuged at 4 °C for 10 min at 480g. The resulting supernatant was centrifuged at 4 °C for 10 min at 30,000g. The pellet was resuspended in 40 ml of Tris-HCl (50 mM), pH 7.5, and MgCl<sub>2</sub> (10 mM), and centrifuged at 4 °C for 10 min at 30,000g. This step was repeated and the final pellet was suspended in 4–6 ml of Tris-HCl (75 mM), pH 7.65, and MgCl<sub>2</sub> (25 mM). This crude particulate preparation was used for binding experiments.

The binding assay was initiated by adding 100  $\mu$ l of the particulate preparation to 50  $\mu$ l of (·)[<sup>3</sup>H]dihydroalprenolol

with and without ( $\pm$ )propranolol ( $10^{-5}$ M final concentration). The mixtures were incubated for 10 min at 37 °C with shaking; 100- $\mu$ l portions were then withdrawn and immediately dispersed in 5 ml of ice-cold Tris-HCl (75 mM), pH 7.65, and  $\text{MgCl}_2$  (25 mM), and filtered by suction through a 25-mm Schleicher and Schuell no. 25 filter disk. The filters were washed four times with 5 ml of buffer, dried and counted. Specific binding was defined as the difference between binding in the absence and presence of ( $\pm$ )propranolol ( $10^{-5}$ M), expressed as fmol bound per mg protein. Specific binding was usually 50–70% of total binding. Dissociation constants ( $K_D$ ) and maximum ( $(-)[^3\text{H}]$ dihydroalprenolol binding were determined from least-squares fits to Scatchard plots of the data<sup>7</sup>.

For experiments using isolated, perfused hearts, rats were killed by guillotine and the hearts rapidly excised. Both atria were removed and the heart was attached to a Langendorf perfusion apparatus by means of a glass cannula and a short piece of rubber tubing. The perfusion buffer was a Krebs-bicarbonate solution containing NaCl (118 mM), KCl (4.8 mM),  $\text{CaCl}_2$  (2.5 mM),  $\text{MgSO}_4$  (1.2 mM),  $\text{KH}_2\text{PO}_4$  (1 mM), glucose (11.1 mM) and  $\text{NaHCO}_3$  (27.2 mM). This solution was maintained at 37 °C and equilibrated with 95% oxygen–5%  $\text{CO}_2$ . The perfusion rate was maintained at 10 ml  $\text{min}^{-1}$ . A suture was placed through the apex of the heart and attached to a strain gauge. The diastolic tension was set at 5 g and the heart was electrically driven at 300 beats  $\text{min}^{-1}$  using monophasic square wave pulses of 10 ms duration at a voltage of 2.5–3.0 V (approximately twice threshold). The paced hearts were perfused for 15 min before isoprenaline administration. Isotonic saline containing ( $\pm$ )isoprenaline was administered at a rate of 0.25 ml  $\text{min}^{-1}$  through a needle inserted just above the glass cannula. The isoprenaline concentrations in the final perfusates were 0,  $10^{-8}$  and  $10^{-7}$ M. After 45 s of drug infusion, hearts were rapidly frozen using Wollenberger tongs precooled in liquid nitrogen. The frozen samples were stored at  $-100$  °C and assayed within 7 d.

**Fig. 1** Scatchard plot of ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding to crude particulate fractions from control (●) and guanethidine-treated (○) rats. Groups of four control and four treated rats were killed by cervical dislocation, the cardiac ventricles rapidly excised and the particulate fraction prepared as described in the text. Specific binding was defined as the difference between binding in the absence and presence of ( $\pm$ )propranolol  $10^{-5}$ M, expressed as fmol bound per mg protein. Specific binding was usually 50–70% of total binding. Data points are means of closely agreeing duplicate observations.



**Table 1** Maximum specific binding and dissociation constants ( $K_D$ ) of ( $-$ ) $[^3\text{H}]$ dihydroalprenolol in crude particulate fractions from control and guanethidine-treated rats

| Treatment group | No. of groups | ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding |                            |
|-----------------|---------------|--------------------------------------------------|----------------------------|
|                 |               | Maximum specific binding (fmol per mg protein)   | Dissociation constant (nM) |
| Control         | 4             | $33.5 \pm 4.2$                                   | $6.05 \pm 0.50$            |
| Guanethidine    | 4             | $55.6 \pm 6.4^*$                                 | $8.52 \pm 1.38$            |

The fractions were prepared and ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding assays carried out as described in the text. Dissociation constants and maximum ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding were determined from least-squares fits to Scatchard plots of the data. The results are the means  $\pm$  s.e.m. of four experiments. Statistical analyses were carried out by Student's *t*-test (unpaired).

\* $P < 0.001$ .

Individual frozen hearts were powdered in a percussion mortar precooled in liquid nitrogen. A small sample was transferred to 2 ml of ice-cold 0.4 N  $\text{HClO}_4$  and homogenised (Polytron PT 10-ST tissue disruptor) at setting 4 for 15 s. The resulting homogenate was centrifuged for 20 min at room temperature at 18,000g. One ml of the supernatant was removed and used for cAMP radioimmunoassay according to the method of Steiner *et al.*<sup>8</sup>. The precipitate was solubilised in 1 M NaOH and the protein determined according to the method of Lowry *et al.*<sup>9</sup>. The data are presented as the mean  $\pm$  s.e.m. and statistical analyses were made using Student's *t*-test for unpaired data;  $P < 0.05$  was taken as a significant difference.

Chronic guanethidine treatment for five weeks followed by a four-week washout period resulted in a significant decrease in ventricular noradrenaline content ( $P < 0.02$ ), thus establishing the success of the guanethidine treatment. Cardiac  $\beta$ -adrenergic receptor number was significantly increased in the guanethidine-treated animals (Fig. 1). Maximum specific ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding in the control groups was  $33.5 \pm 2.6$  fmol per mg protein, compared to a maximum binding of  $55.8 \pm 3.2$  fmol per mg in the experimental groups ( $P < 0.001$ ). Neither the dissociation constant for ( $-$ ) $[^3\text{H}]$ dihydroalprenolol (Table 1) nor the total membrane protein content were significantly different between the two groups. Thus, there were significantly more  $\beta$ -adrenergic receptors in ventricular particulate fractions from the treated animals.

The results obtained using isolated, perfused hearts obtained from control and guanethidine-treated adult animals are presented in Table 2. In control and treated hearts not perfused with isoprenaline, there was no difference in cAMP levels (Table 2). In hearts perfused with  $10^{-8}$ M isoprenaline, cAMP levels were significantly higher in the guanethidine-treated group than in controls (7.98 pmol per mg protein compared with 6.48;  $P < 0.01$ ). However, using  $10^{-7}$ M isoprenaline in the perfusate, there was no significant difference in cAMP between the control and guanethidine-treated animals.

Although several studies of the effects of catecholamine depletion on cardiac tissue have been reported<sup>10,11</sup>, none has measured  $\beta$ -adrenergic receptor number. Our results demonstrate that in chronic guanethidine-treated rats, there is a decrease in cardiac noradrenaline stores and an increase in cardiac  $\beta$ -adrenergic receptors, as assayed by ( $-$ ) $[^3\text{H}]$ dihydroalprenolol binding.

cAMP accumulation in the isolated, perfused heart in response to the  $\beta$ -adrenergic receptor agonist isoprenaline was used as a parameter of agonist stimulation. Isoprenaline was chosen as the agonist because it does not undergo presynaptic reuptake<sup>12</sup>. The choice of isoprenaline is an important one, as supersensitivity noted in response to  $\beta$ -agonists which do undergo reuptake may be related to increased postsynaptic concentrations associated with decreased neuronal uptake rather than true postsynaptic supersensitivity.

Basal cAMP levels in the absence of isoprenaline were not significantly different in the control and experimental hearts. These data suggest that any difference in residual catecholamines



**Table 2** cAMP response to isoprenaline in isolated, perfused hearts from control and guanethidine-treated rats

| Treatment group | cAMP accumulation in response to isoprenaline<br>(pmol per mg protein) |                      |                      |
|-----------------|------------------------------------------------------------------------|----------------------|----------------------|
|                 | Isoprenaline concentration                                             |                      |                      |
|                 | 0                                                                      | $10^{-8}$ M          | $10^{-7}$ M          |
| Control         | $3.19 \pm 0.27$ (4)                                                    | $6.48 \pm 0.37$ (5)  | $9.73 \pm 0.32$ (4)  |
| Guanethidine    | $3.05 \pm 0.09$ (4)                                                    | $7.98 \pm 0.23^*(5)$ | $10.12 \pm 0.47$ (4) |

Control and treated rats were killed by guillotine and the hearts rapidly excised. Both atria were removed and each heart was attached to a Langendorff perfusion apparatus by means of a glass cannula; perfusion experiments were carried out as described in the text. Numbers in parentheses are the number of hearts in each group. \* $P < 0.01$ .

between the control and experimental hearts are not significantly reflected in cAMP levels. Increases in cAMP levels were noted in both the control and experimental hearts when the isoprenaline concentration was raised from 0 to  $10^{-8}$  to  $10^{-7}$  (Table 2). The cAMP levels were significantly increased in the experimental hearts compared to controls using  $10^{-8}$ M isoprenaline, whereas no significant difference was observed at  $10^{-7}$ M isoprenaline. An analogous effect was seen in experiments in which the increase in cAMP in response to noradrenaline was measured in the cerebrum of rats treated with 6-hydroxydopamine<sup>9</sup>. In these experiments, submaximal concentrations of noradrenaline produced an apparent supersensitive response in 6-hydroxydopamine-treated rats, but high concentrations of the catecholamine did not. In these studies,  $10^{-7}$ M isoprenaline presumably stimulates cAMP accumulation in the perfused rat heart maximally whereas  $10^{-8}$ M is a submaximal dose. These data suggest an increase in the 'apparent affinity' of the agonist for stimulating cAMP levels. We did not directly assess the ability of isoprenaline to compete for the binding sites in radioligand binding assays in the treated animals, and hence do not know if any alterations in the affinity of the receptors for isoprenaline occurred. As demonstrated above, however, there is no change in affinity of the receptors for (–) [<sup>3</sup>H] dihydroalprenolol. There is, nevertheless, no difficulty in reconciling an increase in receptor number with an increase in apparent affinity for stimulation of a biological process. This, in fact, would be the predicted result if the number of receptors were not the limiting factor in the maximal biological response ('spare receptors').

In our model, decreased receptor occupancy by agonist resulted in an increased number of  $\beta$ -adrenergic receptors and increased  $\beta$ -adrenergic responsiveness. These results support the hypothesis that there is a dynamic and inverse relationship between receptor occupancy by agonist and receptor number. Further, the changes in receptor number noted in the present experiments are in the same direction as and may be related to the changes in the biochemical responsiveness to  $\beta$ -agonists.

GEORGE GLAUBIGER  
BIE SHUNG TSAI  
ROBERT J. LEFKOWITZ

Departments of Medicine and Biochemistry,  
Duke University Medical Center,  
Durham, North Carolina 27710

BENJAMIN WEISS

Department of Pharmacology,  
Medical College of Pennsylvania,  
Philadelphia, Pennsylvania 19129

EUGENE M. JOHNSON, JR

Department of Pharmacology,  
Washington University School of Medicine,  
St. Louis, Missouri 63110

Received 9 November 1977; accepted 27 March 1978.

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## Spontaneous firing of hypothalamic neurones over a narrow temperature interval

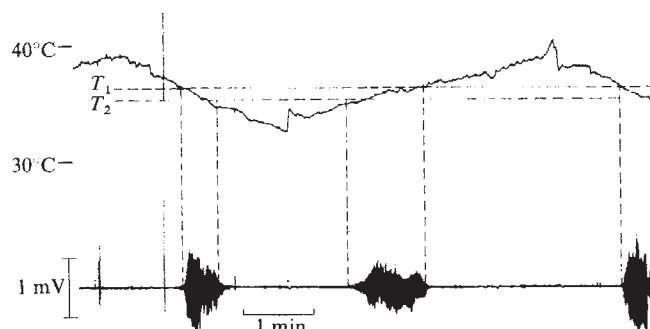
THE search for the central mechanism of thermoregulation in mammals has been narrowed down to a study of neurones in particular regions of the hypothalamus<sup>1,2</sup>. The experimental work has mainly been carried out *in vivo* and thus reflects not only the properties of a single neurone but also the influence of the neuronal network in which it is connected. We are currently measuring spontaneous electrical potentials in dissociated hypothalamic cells grown in culture to determine the thermal behaviour of individual neurones freed from their original synaptic connections. Our primary intention was to measure the temperature-dependence of the firing rate and relate it to published *in vivo* data<sup>1,3</sup>, but our initial experiment produced the unexpected result that a significant proportion of hypothalamic neurones that survive in culture are silent between 30 and 40 °C except inside a narrow temperature range, only about 2° wide, where they show spontaneous electrical activity.

The hypothalamus was dissected from 1-week post partum Sprague–Dawley albino rats and divided into anterior, central and posterior sections. Each section was mechanically dispersed in Hanks' balanced salt solution and the resulting cell suspension was cultured at 37 °C for 16 d. For control purposes, cultures were also prepared from the cerebellum of the same rats.

Optical microscopy during culture showed the initially dispersed cells developing a wide range of states of aggregation. Scanning electron microscopy revealed occasional isolated cells after testing, but the majority were associated into groups with apparently interconnecting processes.

Activity was recorded using borosilicate glass microelectrodes filled with 3 M KCl or NaCl and having impedances in the range 10–50 M $\Omega$ . Only a few intracellular measurements were made, as even with extracellular recording it was difficult to maintain a neurone in its original active condition through several temperature cycles over many minutes. Temperature was controlled using a small

**Fig. 1** Part of recorder trace showing temperature (upper trace) and spontaneous extracellular potential (lower) for a cell in a culture grown from the anterior hypothalamus of a rat.  $T_1$  and  $T_2$  indicate the temperature limits within which firing occurs. From a culture grown at 37 °C for 16 d in 80% medium 199 with DGP, 20% foetal calf serum, glucose 5 mg ml<sup>-1</sup>, penicillin 100 U ml<sup>-1</sup>, insulin 50 mU ml<sup>-1</sup>, 1 mM CaCl<sub>2</sub> and NaHCO<sub>3</sub> to adjust to pH 7.3.



**Table 1** Spontaneous electrical behaviour of neurones

|              | Activity solely in narrow temperature band | Activity additional to narrow temperature band | Activity quite unrelated to temperature | Total number of neurones |
|--------------|--------------------------------------------|------------------------------------------------|-----------------------------------------|--------------------------|
| Hypothalamus | 6                                          | 22                                             | 7                                       | 35                       |
| Cerebellum   | 0                                          | 5                                              | 18                                      | 23                       |

d.c. heating coil and measured using a thermocouple approximately 1 mm from the tip of the microelectrode. The outputs of microelectrode (pre-amplified) and thermocouple were fed to a two-channel chart recorder.

All cells showed a basic noise level which varied up to a few tenths of a millivolt depending on the particular microelectrode. At an appropriate temperature many cells showed spontaneous activity with an amplitude of about 2 mV during extracellular recording; intracellular levels were observed up to about 45 mV. At the slow recording speeds used, this 'activity' can be seen as the envelope of many rapidly changing signals, as shown in Fig. 1. In the present experiments we did not usually resolve the activity further, but examination of some outputs with an oscilloscope or a transient recorder showed the main signals to be spike potentials with half-widths of approximately 3 ms and frequencies of the order of 40 Hz.

The unexpected behaviour was a continuous electrical activity confined within a range of 2–3 °C and centred on a temperature of about 36 °C; this central temperature varied a little from cell to cell, but there was little hysteresis in cycles of heating and cooling. Figure 1 reproduces part of a chart recording of the temperature and the extracellular output from a cell which was spontaneously active in this fashion. Threshold temperatures for the onset and cessation of firing are indicated by  $T_1$  and  $T_2$  respectively. One hardy cell from the anterior hypothalamus withstood eight cycles of heating and cooling before losing its activity. For that cell the mean upper threshold temperature was 37.7 °C and the lower 36.1 °C with a standard error of 0.6 °C.

We emphasise that only a minority (about 20%) of the 35 hypothalamic neurones examined behaved exactly in this way. A larger number showed additional activity at other temperatures, and a further group was active at periods which bore no systematic relationship to the temperature whatsoever. Altogether, electrical activity was localised to particular temperatures in 80% of the hypothalamic neurones but only 22% of the cerebellar controls. The relative proportions of hypothalamic and cerebellar cells in the three categories can be judged from Table 1: a  $\chi^2$  test on this contingency table showed the differences to be significant at better than the 0.1% level. There was no significant difference between the 23 anterior cells and the posterior, so these results were pooled.

The on-off behaviour limited to a single temperature band only appeared in hypothalamic neurones. This would potentially qualify them as thermostatic switching elements in the thermoregulatory process, although on the present evidence we do not of course claim that this actually is their function. Thermoregulation could equally well derive from a particular function of the activity, such as the increase in spike frequency with temperature already reported for rat cerebellum in neurones<sup>3</sup>.

PETER MASON  
HELEN HASAN  
MILENA VALIS

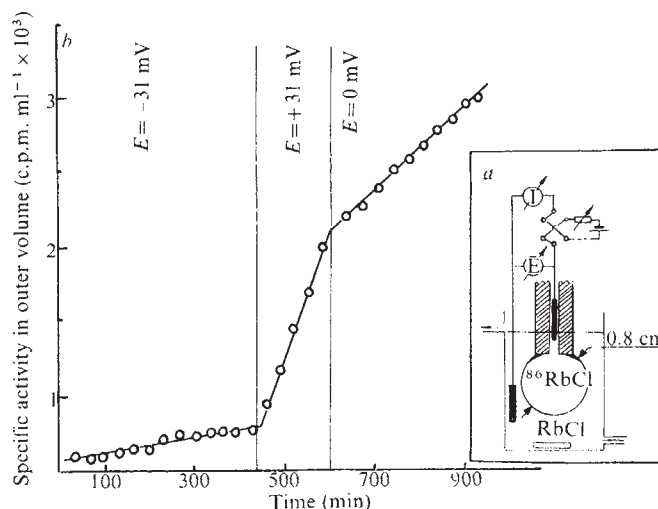
School of Mathematics and Physics,  
Macquarie University, North Ryde,  
New South Wales 2113, Australia

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## Interaction of cation fluxes in gramicidin A channels in lipid bilayer membranes

CLASSICAL studies of potassium movement across the membrane of poisoned giant nerve fibres have shown that passive cationic fluxes through potassium conducting channels are not independent, and suggested a single-file diffusion mechanism<sup>1</sup>. Interaction of ionic fluxes may provide explanations for important properties of cell membranes, such as non-constancy of ionic selectivity coefficients and blocking effects. On the other hand, the characterisation of the interaction of ionic fluxes may provide information on the mechanism of ion movement across membranes. For model membranes with narrow channels formed by compounds of a known chemical structure such as gramicidin A<sup>2–4</sup>, where interaction of ionic fluxes and single-file diffusion should be predicted merely by the pore diameter (about 4 Å), no studies of these phenomena have yet been made. We present here results of the determination of interaction between bidirectional Rb<sup>+</sup> fluxes in gramicidin A channels which penetrate lipid bilayer membranes.



**Fig. 1a.** Arrangement used for the measurement of <sup>86</sup>Rb efflux (explained in text). **b.** A typical plot of specific activity changes (in outer volume) with time at various transmembrane potentials ( $E = E_{\text{inside}} - E_{\text{outside}}$ ).

Long lasting spherical bilayer membranes of surface area up to 2 cm<sup>2</sup> used for the measurements of Rb<sup>+</sup> fluxes were made of bulk ox brain lipids with the addition of cholesterol in a 1:1 (w/w) ratio<sup>5</sup>. The 0.1 or 0.01 M RbCl solutions containing <sup>86</sup>Rb with a specific activity of 30  $\mu\text{Ci ml}^{-1}$  were inside the sphere, while external RbCl solutions were of the same concentration as that inside but initially contained no tracer. Gramicidin A dissolved in ethanol was added to the outside solution up to a final concentration between 10<sup>-9</sup> and 10<sup>-7</sup> M. Controlled potential difference across the membrane varied from 0 to 54 mV. The specific conductance of membranes in various experiments ranged from  $5.8 \times 10^{-8}$  to  $1.6 \times 10^{-5} \Omega^{-1} \text{cm}^{-2}$ .

Investigation of bi-ionic potentials and current-voltage characteristics both in symmetrical and asymmetrical electrolyte conditions, as well as the determination of conductance block-



ing produced by the addition of a small amount of  $\text{TI}^+$  ions, showed that gramicidin A-modified bulk brain lipid bilayers demonstrate almost the same properties as glycerol mono-oleate bilayers<sup>6</sup>.

The efflux of Rb was calculated from the rate of gain of tracer Rb by the external solution<sup>5</sup>. A typical plot of the number of counts against time at various potential differences is shown in Fig. 1.

Fluxes of  $\text{Rb}^+$  measured at transmembrane potentials of opposite signs allowed the determination of the transference number for  $\text{Rb}^+$  from the ratio<sup>7</sup>:

$$t_{\text{Rb}} = [zF(\Phi_{+E} - \Phi_{-E})]/I \quad (1)$$

where  $\Phi_{+E}$  and  $\Phi_{-E}$  are the Rb effluxes measured at transmembrane potentials  $+E$  and  $-E$ ,  $z$  is the charge of cation,  $F$  is the Faraday constant and  $I$  is the transmembrane current. The result of nine experiments has shown that  $t_{\text{Rb}} = 1.00 \pm 0.06$  (mean  $\pm$  s.d.). Hence, it has been concluded that only cations can pass through gramicidin A channels. These data are in accordance with those we obtained on electrochemical determination of cation transference number for gramicidin A channels and with those previously published<sup>8</sup>.

In our investigations of the interaction of bidirectional fluxes in gramicidin A channels, we used the approach developed by Hodgkin and Keynes<sup>1</sup>. In terms of this approach, the interaction of fluxes in the pore might be assumed when, in the modified Ussing equation<sup>1</sup>,

$$\Phi_{+E}/\Phi_{-E} = \exp(nzFE)/(RT) \quad (2)$$

the index  $n$  exceeds unity. In the case of single-file diffusion and a complete occupancy of sites in the channels, the index  $n$  gives the number of ions simultaneously present in the channel<sup>1,9</sup>.

The results of 13 experiments used for determination of the  $n$  value from equation (2) are presented in Table 1. The value  $n$  found in the whole range of the above mentioned experimental conditions was apparently close to 2 ( $1.94 \pm 0.17$ ).

The value of index  $n$  can also be determined from the equation<sup>1</sup>

$$G_o = F^2 \Phi_o n / RT \quad (3)$$

where  $\Phi_o$  is  $\text{Rb}^+$  flux and  $G_o$  is the membrane conductance measured at the zero potential difference across membranes. The mean value of  $n$  ( $1.98 \pm 0.24$ ) calculated from equation (3) in a series of 17 experiments (Table 2) agreed well with that obtained by the first method.

Differences between the  $n$  values and unity in both series were statistically significant, and, therefore, bidirectional

fluxes in the gramicidin A channels should be considered non-independent. In the case of complete sites occupancy,  $n = 2$ , means that two cations are simultaneously present in the channel. In this respect the system studied differs from amphotericin B and nystatin treated membranes, where the index  $n$  seemed to be equal to unity<sup>7</sup>, possibly because of a greater diameter (7–8 Å) of pores formed in bilayers by polyene antibiotics. Even more pronounced differences are observed between the  $n$  values if one compares the results presented here for gramicidin A treated membranes with those for bilayers modified by valinomycin<sup>10</sup>, where the index  $n$  in certain conditions was found to be as low as 0.5. The latter might be considered as evidence of the involvement of an exchange diffusion mechanism in ion transport.

The simplest calculation of conductivity of single gramicidin A channel based on the parameters of  $\pi^6_{\text{LD}}$  helix dimer with a pore diameter of 4 Å and pore length of 30 Å gives a value of about  $30 \times 10^{-12} \Omega^{-1}$ , provided that the mobility and concentration of cations in the pore are the same as those in adjoining water solutions. The above value is close to that of the conductivity of a single gramicidin A channel in bilayers ( $\sim 10 \times 10^{-12} \Omega^{-1}$ ) (ref. 11). It is reasonable to assume a lower ionic mobility

**Table 2** Values of  $n$  calculated from the ratio of membrane conductance ( $G_o$ ) to Rb flux ( $\Phi_o$ ) at zero potential difference (equation (3))

| RbCl concentration (M) | $G_o$ ( $\Omega^{-1} \text{cm}^{-2} \times 10^7$ ) | $\Phi_o$ ( $\text{pM cm}^{-2} \text{s}^{-1}$ ) | $n$                         |
|------------------------|----------------------------------------------------|------------------------------------------------|-----------------------------|
| 0.01                   | 0.58                                               | 0.0084                                         | 1.8                         |
| 0.01                   | 0.94                                               | 0.013                                          | 1.9                         |
| 0.01                   | 2.6                                                | 0.029                                          | 2.4                         |
| 0.01                   | 5.0                                                | 0.061                                          | 2.2                         |
| 0.01                   | 7.6                                                | 0.095                                          | 2.1                         |
| 0.01                   | 9.7                                                | 0.15                                           | 1.7                         |
| 0.1                    | 8.6                                                | 0.14                                           | 1.6                         |
| 0.1                    | 11                                                 | 0.17                                           | 1.7                         |
| 0.1                    | 14                                                 | 0.20                                           | 1.9                         |
| 0.1                    | 17                                                 | 0.22                                           | 2.0                         |
| 0.1                    | 22                                                 | 0.24                                           | 2.4                         |
| 0.1                    | 22                                                 | 0.30                                           | 2.0                         |
| 0.1                    | 30                                                 | 0.38                                           | 2.1                         |
| 0.1                    | 62                                                 | 0.78                                           | 2.1                         |
| 0.1                    | 63                                                 | 0.76                                           | 2.2                         |
| 0.1                    | 99                                                 | 1.5                                            | 1.8                         |
| 0.1                    | 160                                                | 2.5                                            | 1.7                         |
|                        |                                                    |                                                | Mean $1.98 \pm 0.24$ (s.d.) |

in the pore; hence, the concentration of cations within the pore should exceed that in free water solutions in order to adjust calculated and measured single channel conductance. If two  $\text{Rb}^+$  ions are simultaneously present in gramicidin A dimer channel, which follows from the results of the present investigation, the ionic concentration in the pore volume should be about 100 times that in 0.1 M RbCl solution. In this case, the accumulation of  $\text{Rb}^+$  in the cavity of gramicidin A molecule reveals the 1:1 stoichiometry previously found for alkali cation binding by gramicidin A in organic solvents<sup>12</sup>.

Taking into account that the potassium channels in nerve membranes<sup>1,13</sup> and the gramicidin A channels in bilayers<sup>11</sup> have comparable conductivity and similar numbers of cations inside, the gramicidin A treated bilayer membranes might serve as a model for detailed studies on ionic flux interaction phenomena.

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LUDMILA V. SCHAGINA  
A. E. GRINFELDT  
A. A. LEV\*

Laboratory of Physical Chemistry of Cell Membranes,  
Institute of Cytology of the Academy of Sciences  
of the U.S.S.R.,  
Maclina Prospect 32,  
Leningrad, 190121, USSR

**Table 1** Values of the index  $n$  as calculated by equation (2) and the experimental data used for the calculations

| RbCl concentration (M) | $G$ ( $\Omega^{-1} \text{cm}^{-2} \times 10^7$ ) | $E$ (mV) | $\frac{\Phi_{+E}}{\Phi_{-E}}$ | $n$                         |
|------------------------|--------------------------------------------------|----------|-------------------------------|-----------------------------|
| 0.01                   | 2.2                                              | 24       | 5.4                           | 1.8                         |
| 0.01                   | 4.6                                              | 18       | 3.6*                          | 1.8                         |
| 0.01                   | 7.2                                              | 17       | 4.0                           | 2.1                         |
| 0.01                   | 8.2                                              | 54       | 83                            | 2.1                         |
| 0.01                   | 9.2                                              | 30       | 8.4*                          | 1.8                         |
| 0.1                    | 9.8                                              | 42       | 24                            | 1.9                         |
| 0.1                    | 12                                               | 41       | 20                            | 1.8                         |
| 0.1                    | 16                                               | 15       | 3.4*                          | 2.1                         |
| 0.1                    | 16                                               | 30       | 8.7*                          | 1.8                         |
| 0.1                    | 18                                               | 15       | 3.1*                          | 1.9                         |
| 0.1                    | 23                                               | 48       | 31*                           | 1.8                         |
| 0.1                    | 28                                               | 27       | 8.3                           | 2.0                         |
| 0.1                    | 69                                               | 31       | 16                            | 2.3                         |
|                        |                                                  |          |                               | Mean $1.94 \pm 0.17$ (s.d.) |

\*Only  $\Phi_{-E}$  values were measured;  $\Phi_{+E}$  was calculated from equation (1) and established  $t_{\text{Rb}} = 1$ .

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\*To whom correspondence should be addressed.

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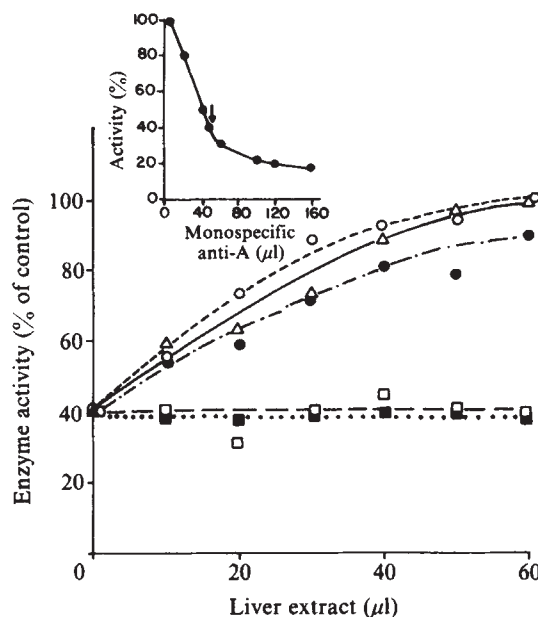
## Altered $\alpha$ subunits in Tay-Sachs disease

SANDHOFF-JATZKEWITZ disease (type O variant), in which both hexosaminidase (Hex) A and B are deficient, has been shown to result from a mutation in the structural gene for subunit ( $\beta$ ) common to both Hex A and B (refs 1, 2). This conclusion was based on the demonstration of the presence of enzymatically inactive protein (crossreacting material, CRM) in the liver samples of patients with Sandhoff disease (SD). In Tay-Sachs disease (TSD), in which only Hex A is deficient, CRM was not demonstrated previously, using antiserum raised against native Hex A and B (refs 2-4). It was, however, suggested that this disorder is also due to a mutation in the structural gene coding for the unique subunit ( $\alpha$ ) of Hex A (refs 1, 2). Using antisera raised against cross-linked human placental Hex A, we have demonstrated and report here the presence of CRM in liver samples from eight unrelated patients with TSD.

Antibodies against homogeneous preparations of human placental Hex A (native), purified as described earlier for the kidney enzyme<sup>5</sup>, and cross linked with glutaraldehyde, were raised in rabbits<sup>2,6</sup>. About 75  $\mu$ g of protein were cross linked with 5% glutaraldehyde and injected intradermally, at multiple sites, with a 2-week interval between injections. The monospecific anti-cross-linked Hex A antiserum was prepared by either passing the antiserum through a column of Hex B insolubilised on beaded Sepharose, or by absorption with a homogeneous preparation of Hex B.

Liver samples for immunological studies were homogenised in a Sorvall omnimixer at a speed of 8,000 r.p.m. for 15 min in 10 mM potassium phosphate buffer, pH 7.0, to make a 10% homogenate. The homogenate was centrifuged at 10,000g for 15 min at 4 °C and the supernatant was concentrated by ultrafiltration. Hex B-free Tay-Sachs liver extracts (TLE) were prepared by passing 4 ml of Tay-Sachs liver supernatant through a column of CM-52 (5 ml bed volume) equilibrated with 10 mM citrate-phosphate buffer, pH 5.0, which adsorbed all of the Hex B activity. The unadsorbed fractions were divided into four equal portions. One portion was designated as Hex B-free TLE: the other three were passed through immunoaffinity (beaded Sepharose) columns of either anti-native Hex A, anti-cross-linked Hex A, or anti-Hex B. The unadsorbed fractions from each column and Hex B-free TLE were tested for the Hex activity by using 4-methyl-umbelliferyl- $\beta$ -D-N-acetyl glucosaminide and for immunological reactivity with anti-Hex B by double immunodiffusion. All of the samples were found to be free of Hex activity and Hex B antigen. To adsorb Hex B from the normal liver extract, it was passed through a CM-52 column in conditions similar to those used for TLE preparation. The unadsorbed fraction was further passed through a column of anti-native Hex A insolubilised on beaded Sepharose. The unadsorbed fraction was found to be free of Hex activity and Hex A and B antigens.

A homogeneous preparation of human placental Hex A was precipitated with varying concentrations of mono-



**Fig. 1** Immunotitrations to demonstrate the CRM in Tay-Sachs liver extract. The subset demonstrates the precipitation of Hex A with the monospecific anti-cross-linked Hex A. Amount of antiserum, as marked by arrow, which precipitated 60% of Hex A activity was used in all the titrations. The incubation mixture (250  $\mu$ l), contained 40  $\mu$ l of monospecific anti-cross-linked Hex A, 50  $\mu$ l of Hex A (150 mU ml<sup>-1</sup>), varying amounts of various Hex B-free liver extracts and 10 mM phosphate buffer containing 4% bovine serum albumin. The samples were incubated overnight at 4 °C, centrifuged at 10,000g for 1 h and supernatant assayed for enzyme activity<sup>5</sup>.  $\Delta$ , Hex B-free Tay-Sachs liver extract (TLE);  $\circ$ , Hex B-free TLE + anti-B;  $\bullet$ , Hex B-free TLE + anti-A (native);  $\square$ , Hex B-free TLE + anti-A (cross linked);  $\blacksquare$ , Hex B-free normal liver extract + anti-A (native).

specific anti-cross-linked Hex A antiserum as described in Fig. 1 (subset). Varying amounts of the normal and Tay-Sachs liver extracts described above were added to a fixed ratio of Hex A: monospecific anti-cross-linked Hex A. This had precipitated approximately 60% of Hex A. The addition of Hex B-free TLE to a mixture of Hex A and monospecific anti-cross-linked Hex A resulted in practically no precipitation of Hex A, whereas in the absence of Hex B-free TLE, about 60% precipitation of Hex A was observed. Similar results were obtained when Hex B-free TLE, adsorbed on insolubilised anti-Hex B or anti-native Hex A columns, was added. These studies indicate that Hex B-free TLE contains a protein that competes with Hex A for the binding sites on the monospecific anti-cross-linked Hex A antibody molecules. This protein is not absorbed by either anti-Hex B or anti-native Hex A antisera, and will be referred to as CRM.

Addition of varying amounts of normal liver extract adsorbed on CM-52 and anti-native Hex A serum, or Hex B-free TLE adsorbed on anti-cross-linked Hex A serum did not have any effect on the precipitation of Hex A by monospecific anti-cross-linked Hex A serum. From these control experiments it can be concluded that: (1) CRM is unique to Tay-Sachs livers, that is, it is absent in normal livers; and (2) demonstration of CRM in Tay-Sachs livers is not due to a nonspecific interference by liver extracts in the precipitation of Hex A with monospecific anti-cross-linked Hex A.

Since antiserum against native Hex A does not detect the CRM in TLE, whereas antiserum against cross-linked Hex A can do so, two possibilities must be considered. The first is that the antigen contained extremely small amounts of contaminating protein which became immunogenic after cross linking. The second possibility is that cross linking of Hex A results in conformational changes that make



certain sites (heterotopes) immunogenic. The antiserum against the modified protein then recognises the CRM. The first possibility can be eliminated, as evidenced in the control experiments. The presence of heterotopes, as demonstrated earlier for other proteins<sup>7-9</sup>, can explain our observations.

The presence of CRM in TLE was also demonstrated by immunoelectrophoresis, double immunodiffusion and radial immunodiffusion studies (to be published). As anti-Hex B does not precipitate the CRM, it is indicated that the altered 'α' subunits in TLE are not combined with 'β' subunits. It may, therefore, be concluded that TSD, like SD, is caused by structural gene mutation and that it may be possible to measure the CRM in the serum and amniotic cells of the hetero- and homozygotes for TSD using radioimmunoassay.

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SATISH K. SRIVASTAVA  
NASEEM H. ANSARI

Department of Human Biological  
Chemistry and Genetics,  
The University of Texas Medical Branch,  
Galveston, Texas 77550

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## Effect of growth hormone on vitamin D metabolism

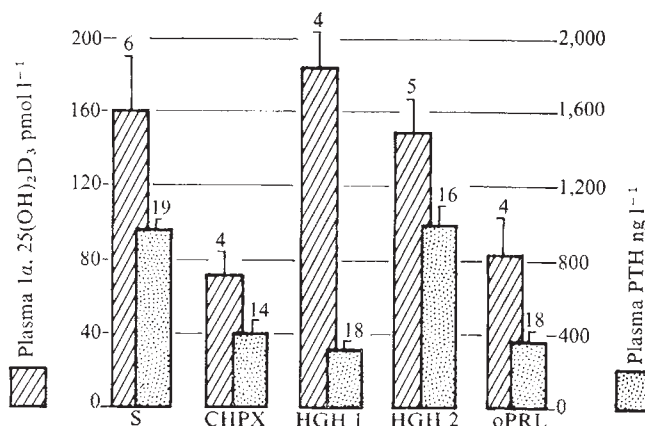
BEFORE affecting calcium and phosphorus metabolism, vitamin D<sub>3</sub> (cholecalciferol) undergoes two hydroxylations; first in the liver, gut and perhaps other organs, to its major circulating form<sup>1,2</sup> 25(OH)D<sub>3</sub> and then in the kidney<sup>3</sup> to 1α,25(OH)<sub>2</sub>D<sub>3</sub>, the hormonal form. 1α,25(OH)<sub>2</sub>D<sub>3</sub> is the most potent metabolite of cholecalciferol and may be chiefly responsible for all the effects of the vitamin on calcium metabolism<sup>4</sup>. Although the regulation of the secretion of 1α,25(OH)<sub>2</sub>D<sub>3</sub> by the kidney has been a controversial subject, it is generally agreed that 1α,25(OH)<sub>2</sub>D<sub>3</sub> itself<sup>5,6</sup>, the calcium and phosphorus content<sup>7-9</sup> of the diet and parathyroid hormone (PTH)<sup>10</sup> are all involved. But these factors do not seem adequate to account for the increased content of 1α,25(OH)<sub>2</sub>D<sub>3</sub> in the plasma during growth spurts, pregnancy and lactation<sup>11</sup>, when the major increases in calcium and phosphorus absorption occur. We thought there might be a clue in the action of prolactin, which strongly increases the secretion of 1α,25(OH)<sub>2</sub>D<sub>3</sub> in birds<sup>12,13</sup> and enhances plasma 1α,25(OH)<sub>2</sub>D<sub>3</sub> in lactating mammals<sup>14</sup>. Suppression of prolactin by administration of bromocriptine to lactating rats markedly lowers plasma 1α,25(OH)<sub>2</sub>D<sub>3</sub> levels, although the drug has no such effect in non-lactating controls<sup>15</sup>. The similarity of the amino acid sequences of prolactin and growth hormone suggested that these hormones might have similar effects. We now report that administration to rats of a highly purified preparation of human growth hormone restores to normal the depressed plasma levels of 1α,25(OH)<sub>2</sub>D<sub>3</sub> produced by hypophysectomy.

Male Wistar rats were hypophysectomised by the trans-sphenoidal route<sup>16</sup>, and, after 8 d of equilibration, were treated for 7 d with either growth hormone or ovine prolactin. Appropriate control groups were also studied. After

7 d of treatment, plasma 1α,25(OH)<sub>2</sub>D<sub>3</sub> was estimated in triplicate by the technique of Brumbaugh *et al.*<sup>17</sup> and plasma parathyroid hormone was measured by radioimmunoassay (Fig. 1).

Compared with sham operation, hypophysectomy produced a considerable decrease in plasma 1α,25(OH)<sub>2</sub>D<sub>3</sub>, which was restored completely by administration of growth hormone. It is interesting that ovine prolactin had no effect in these experimental conditions, although it is fully active in maintaining lactation in bromocriptine-treated rats<sup>18</sup>. These results provide strong evidence that growth hormone enhances calcium and phosphorus absorption during growth by influencing vitamin D metabolism to increase the production of 1α,25(OH)<sub>2</sub>D<sub>3</sub>.

The mechanism of this effect is not clear. Thus, Fig. 1 also shows that plasma PTH decreased markedly after hypophysectomy and that this was reversed only by the larger dose of growth hormone. These findings do not support the possibility that the effects on 1α,25(OH)<sub>2</sub>D<sub>3</sub> were secondary to changes in PTH: 50 μg of growth hormone twice daily produced the full effect on plasma 1α,25(OH)<sub>2</sub>D<sub>3</sub> without altering PTH levels from control. But mediation by PTH cannot be excluded completely because it is just conceivable that transient changes occurred early in the experiment, producing persistent



**Fig. 1** Effect of human growth hormone on plasma levels of 1α,25(OH)<sub>2</sub>D<sub>3</sub> and PTH. Values are mean ± s.e.m.; the number of samples in each group is shown above the columns. To provide adequate volumes for replicate analyses of 1α,25(OH)<sub>2</sub>D<sub>3</sub>, plasma was pooled from subgroups of animals to provide four to six samples in each group: triplicate assays for 1α,25(OH)<sub>2</sub>D<sub>3</sub> were made by the radioreceptor technique<sup>17</sup>. Male Wistar rats (mean weight 212 g before hypophysectomy) were used and fed a diet containing 1% calcium, 0.9% phosphorus and 2 IU vitamin D<sub>3</sub> per g for 4 weeks after weaning. After hypophysectomy, each group was allowed to equilibrate for 8 d with 5% glucose as drinking water for the hypophysectomised groups. The sham-operated group(S) received solvent, but for a further 7 d the other (hypophysectomised) groups received solvent alone (CHPX), human growth hormone 50 μg twice daily (HGH1), human growth hormone 250 μg twice daily (HGH2), or 50 μg twice daily of ovine prolactin, Sigma, 26 IU mg<sup>-1</sup> (oPRL). The effects of hypophysectomy and of both doses of growth hormone are highly significant ( $P < 0.01$ ). The preparation of human growth hormone contained less than 0.1% of other pituitary hormones and had a potency of 2.5 IU mg<sup>-1</sup>. The lower dose (HGH1) is within the range of doses of human growth hormone given daily for 7 d to hypophysectomised rats in growth assays of the human hormone at the National Institute for Biological Control and Standards (personal communication from D. R. Bangham). PTH was measured by radioimmunoassay on plasma samples from individual rats except where insufficient plasma was available. Extracts of rat parathyroid tissue produced displacement curves indistinguishable from those due to human PTH. The results are expressed in terms of MRC human parathyroid hormone standard (reference 75/549). The changes in PTH due to hypophysectomy (CHPX) and to 250 μg HGH twice daily are highly significant ( $P < 0.01$ ). 50 μg HGH twice daily produced marked elevation in 1α,25(OH)<sub>2</sub>D<sub>3</sub> without any change in PTH levels.

Table 1 Effect of growth hormone in hypophysectomised rats

|                                                   | Plasma calcium<br>(mmol l <sup>-1</sup> ) | Plasma phosphate<br>(mmol l <sup>-1</sup> ) | Mean weight<br>gain (g) | Plasma 1 $\alpha$ ,25(OH) $_2$ D $_3$<br>(pmol l <sup>-1</sup> ) |
|---------------------------------------------------|-------------------------------------------|---------------------------------------------|-------------------------|------------------------------------------------------------------|
| Sham                                              | 2.91 $\pm$ 0.03 (6)                       | 2.80 $\pm$ 0.17 (6)                         | 27.8 $\pm$ 1.6 (20)     | 160 $\pm$ 31 (6)                                                 |
| Control hypophysectomised                         | 2.73 $\pm$ 0.05 (4)                       | 2.03 $\pm$ 0.06 (4)                         | -2.0 $\pm$ 0.9 (21)     | 72 $\pm$ 12 (4)                                                  |
| Human growth hormone 50 $\mu$ g $\times$ 2 per d  | —*                                        | —*                                          | 23.3 $\pm$ 1.3 (21)     | 184 $\pm$ 19 (4)                                                 |
| Human growth hormone 250 $\mu$ g $\times$ 2 per d | 2.83 $\pm$ 0.09 (5)                       | 2.34 $\pm$ 0.13 (5)                         | 30.0 $\pm$ 1.0 (21)     | 148 $\pm$ 17 (5)                                                 |
| Ovine prolactin 50 $\mu$ g $\times$ 2 per d       | 2.71 $\pm$ 0.06 (4)                       | 1.89 $\pm$ 0.17 (4)                         | -0.3 $\pm$ 1.1 (20)     | 81 $\pm$ 21 (4)                                                  |

Plasma calcium and phosphate values compared with weight gains and 1 $\alpha$ ,25(OH) $_2$ D $_3$  levels. As detailed in the legend to Fig. 1, plasma samples were from pooled subgroups of each experimental group. All values are mean  $\pm$  s.e.m. (number of samples). The changes in plasma 1 $\alpha$ ,25(OH) $_2$ D $_3$  levels produced by hypophysectomy and by administration of growth hormone are both highly significant ( $P < 0.01$ ).

\* Calcium and phosphate were not measured in this group to conserve plasma.

alterations in 1 $\alpha$ ,25(OH) $_2$ D $_3$ . It also seems unlikely that the alterations in vitamin D metabolism are mediated by changes in calcium and phosphorus: plasma phosphate was increased to levels close to those of the sham-operated group by growth hormone, and the plasma calcium increased slightly (Table 1); both changes would tend to depress, rather than increase, production<sup>6,7</sup> of 1 $\alpha$ ,25(OH) $_2$ D $_3$ . One possible intermediary of the effect, however, is somatomedin. This hypothesis may be rendered more plausible by recent findings: somatomedin and insulin are structurally related<sup>18</sup>, and insulin can increase 1 $\alpha$ ,25(OH) $_2$ D $_3$  in diabetic rats<sup>19</sup>. The lack of effect of prolactin is interesting because, together with the demonstration that this hormone has a strong effect in chicks and in certain conditions in mammals, it confirms that prolactin acts only in conjunction with other factors, and in mammals only during lactation<sup>12-15</sup>.

Our results emphasise further that the regulation of vitamin D metabolism is extremely complex. But it now seems clear that the major physiological changes in calcium absorption which occur in lactation and growth depend on changes in plasma 1 $\alpha$ ,25(OH) $_2$ D $_3$ . Further, it is possible that these alterations depend, at least in part, on prolactin and growth hormone, respectively. It is tempting to suggest that the similar changes found in pregnancy involve placental lactogen which is closely structurally related to growth hormone. It also now seems possible that the change in calcium absorption in acromegaly in man<sup>20</sup> is due to increased production of 1 $\alpha$ ,25(OH) $_2$ D $_3$ . In any event, we believe our results strongly suggest that growth hormone is a major determinant of vitamin D metabolism in health.

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E. SPANOS  
D. BARRETT  
I. MACINTYRE

Endocrine Unit,  
Royal Postgraduate Medical School,  
Ducane Road, London W12, UK

J. W. PIKE  
E. F. SAFILIAN  
M. R. HAUSSLER

Department of Biochemistry,  
University of Arizona College of Medicine,  
Tucson, Arizona 85721

Received 25 July 1977; accepted 17 March 1978.

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## Structure of oxymyoglobin

MYOGLOBIN (Mb) consists of a single polypeptide chain of 153 residues and one haem. It combines reversibly with molecular oxygen which it takes up from the blood and passes on to mitochondria in muscle. *In vivo* its iron atom remains ferrous, but *in vitro* it autoxidises to the ferric metmyoglobin (metmb) in which the sixth ligand at the iron is water. Metmb of sperm whale was the first protein structure to be determined<sup>1</sup>. Takano recently determined the closely related structure of deoxymb by X-ray analysis, while that of COmb has been determined by neutron diffraction (refs 2, 3 and B. P. Schoenborn, personal communication). The most interesting structure, that of oxymb, has proved elusive. Watson and Nobbs tried to determine it by rapid crystallisation and collection of X-ray data at 4 °C, but even so autoxidation obscured the results<sup>4</sup>. Recent advances in low temperature techniques encouraged me to try again, especially in view of the wide interest in the nature of the iron-oxygen bond<sup>5-7</sup>.

Sperm whale oxymyoglobin failed to crystallise, except for one occasion. Deoxymb<sup>2</sup> was therefore crystallised; the crystals were washed and exposed to air immediately before mounting on the X-ray diffractometer. During collection of diffraction data the crystals were cooled to -12 °C in a cold airstream. Cooling below this temperature cracked them. After 5 d in the X-ray beam there was negligible reduction in X-ray intensities and little evidence of oxidation. After exposure, each crystal was dissolved and its visible spectrum recorded. Data from those showing more than a trace of metmb were discarded.

Diffraction data were collected to a resolution of 2.0 Å, giving a total of 9,181 independent reflections after processing and removal of unreliable observations. *R*-factors (on structure

Table 1 Iron-oxygen coordination geometry

|                       | Fe-O (Å)      | O-O (Å) | Fe-O-O (deg) |
|-----------------------|---------------|---------|--------------|
| Picket-fence complex* | 1.75 (ref. 2) | 1.17    | 129          |
|                       |               | 1.15    | 133          |
| Oxymb†                | 1.9           | 1.4     | 121          |

\*Picket-fence values may be affected by disorder mentioned in text.

†These values are preliminary and may change with further refinement. The bond angle is the only parameter not constrained.



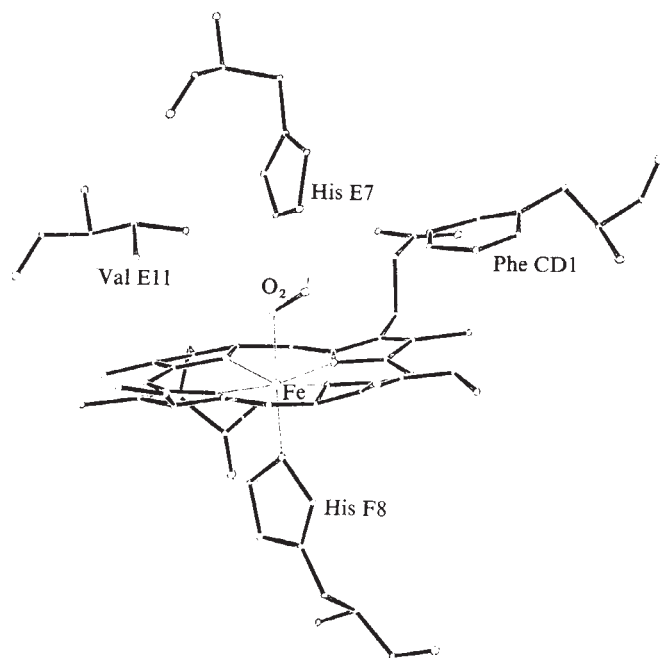


Fig. 1 The haem group and oxygen environment in oxy-myoglobin.

amplitude) and difference electron density maps were calculated using Takano's amplitudes and phases for metmb\* ( $R = 12.2\%$ ) and deoxyhb\* ( $R = 13.8\%$ ). Both maps show peaks corresponding to the coordinated dioxygen ligand in the bent, end-on geometry first proposed by Pauling<sup>5</sup>. The ligand orientation is consistent with the peak observed by Watson and Nobbs in their (oxy-met) difference map. The maps also show widespread small changes in structure similar in magnitude to those found between met and deoxyhb\*<sup>2-8</sup>. A model of the oxyhb structure was built, based on the metmb structure, and refined using a constrained reciprocal-space least-squares procedure developed by A. Jack (personal communication). The current conventional  $R$  value is 20%. Refinement has not yet converged, but further cycles have been postponed until higher resolution data have been collected. Figure 1 shows the current model of the haem group and its immediate environment. Figure 2 shows the peak in the difference Fourier map produced using  $|F|_{\text{calc}}^{\text{oxy}} - |F|_{\text{calc}}^{\text{deoxy}}$  as coefficients and  $\alpha_{\text{calc}}^{\text{oxy}}$  as phase angles, but omitting the oxygen molecule from the structure factor calculation. The integrated electron density in the peak suggests that the site is about 90% occupied.

Collman's 'picket-fence' complex<sup>9,10</sup>, the only oxygen adduct of an iron porphyrin whose structure is known and can therefore

Fig. 2 Section through the Fe-O-O plane of the difference map calculated with oxygen not included in the structure factor calculation. Contours are  $0.15 \text{ e } \text{\AA}^{-3}$  with zero level omitted and negative ones dotted.

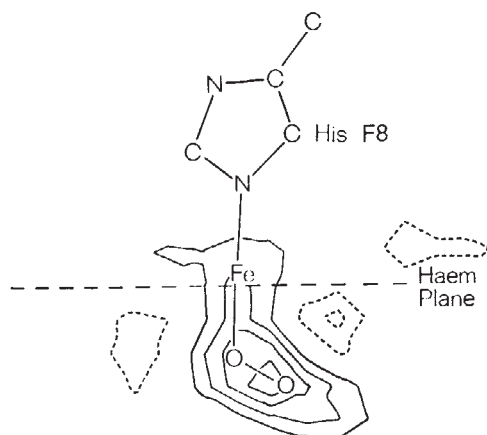


Table 2 Haem geometry in Mb and Hb derivatives

|                                   | Deoxyhb  | Deoxyhb      | Metmb    | Methb        | Oxyhb | COMb*    |
|-----------------------------------|----------|--------------|----------|--------------|-------|----------|
| Fe-(haem plane)                   | 0.55     | 0.60<br>0.63 | 0.40     | 0.07<br>0.21 | 0.33  | 0.24     |
| F8 (N <sub>E</sub> )-(haem plane) | 2.6      | 2.6<br>2.8   | 2.5      | 2.2<br>2.4   | 2.3   | 2.4      |
|                                   | (ref. 2) | (ref. 11)    | (ref. 8) | (ref. 12)    |       | (ref. 3) |

Values are given in Ångstrom units.

\*Calculated using preliminary coordinates from the current, incompletely refined model.

serve as a model for oxyhb, has bent oxygen coordination with the geometry shown in Table 1. The iron lies displaced by  $0.015(3) \text{ Å}$  from the mean porphyrin plane towards the dioxygen ligand. The oxygen takes up four alternative configurations, each with the Fe-O-O plane bisecting the N-Fe-N lines in the porphyrin. The Fe-O-O angles are  $129^\circ$  and  $133^\circ$ . The axial base, 1-methylimidazole, is similarly disordered, its plane making an angle of  $20^\circ$  with N-Fe-N. The oxyhb geometry is somewhat different. The iron is displaced from the mean porphyrin plane by  $0.33 \text{ Å}$  towards the proximal His F8. The oxygen is ordered, with Fe-O-O =  $121^\circ$ , close to the ideal  $120^\circ$ . The imidazole of His F8 and Fe-O-O are approximately coplanar and eclipse the Fe-N (pyrrole II) bond. N<sub>E</sub> of His F8 lies  $2.3 \text{ Å}$  from the mean porphyrin plane.

A staggered arrangement of the imidazole and the Fe-O-O plane relative to N-Fe-N, as found in the picket-fence complex, is favoured because it minimises van der Waals repulsion and maximises overlap of the Fe d orbitals with the  $\pi$  orbitals of the oxygen and imidazole nitrogen. In oxyhb the imidazole is directly away from this orientation, and the oxygen is constrained to the eclipsed orientation by steric hindrance of the distal residues Phe CD1, Val E11 and His E7. The displacement of the iron from the mean porphyrin plane by  $0.33 \text{ Å}$  is a little larger than the  $0.24 \text{ Å}$  found in COMb (Table 2), but the difference is probably not significant at this resolution.

The results show that the transition from deoxy to oxyhb is associated with a movement of the iron atom and the proximal histidine towards the porphyrin plane, but the magnitude of the movement is smaller than expected, as is the very small shift in going from the high-spin met to the low-spin oxyhb. In haemoglobin, where the transition from the liganded to the unliganded form is accompanied by a change in quaternary structure, the shifts in going from deoxy to met are much greater ( $0.4\text{--}0.5 \text{ Å}$ ) so that the shift in going from deoxy to oxyhb must be correspondingly greater.

The parameters of oxyhb here are preliminary and subject to refinement at higher resolution, but they clearly show that the oxygen is ordered and bent to the haem normal, and that the iron atom does move towards the porphyrin plane on oxygenation.

I thank Dr M. F. Perutz for stimulating discussions and Dr B. P. Schoenborn for his preliminary coordinates of COMb.

SIMON E. V. PHILLIPS

MRC Laboratory of Molecular Biology,  
Hills Road, Cambridge, UK

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# reviews

## Physiology of *Octopus*

J. B. Messenger

*Octopus: Physiology and Behaviour of an Advanced Invertebrate.* By M. J. Wells. Pp. 417. (Chapman and Hall: London, 1977.) £17.

LONG before J. Z. Young and his colleagues began the detailed investigations of the nervous system that have recently made the octopus so famous, physiologists had studied its heart rate and colour change mechanism, its blood and its reproductive system. There is an extensive recent literature, too, on many aspects of non-nervous physiology and Dr Wells has attempted to review all this and set it in the context of the behaviour of the animal. He has also re-examined the now considerable body of work on the brain and sense organs.

Although he states that his aim has been "to produce a comprehensive physiology of a single invertebrate type" there are bound to be doubts about how far he has achieved this. And these doubts will not be allayed by his cavalier confession that: "I have never, personally, been able to find much excitement in chemistry and I have cravenly evaded discussion at levels where I am frankly incompetent and reluctant to do the necessary homework". (Ammunition for tutorial students here.) "A monograph . . . is bound to be skewed by too much print squandered on things that interest the author, and too little on the things that may interest others. I am sorry about this. But not very". It should be said at once, however, that this book will be extremely useful for an undergraduate, for a postgraduate beginning his research into cephalopods, or for anyone anxious to find his way into the literature; the work is well documented and there is an extensive reference list. Whether such people will themselves lay out £17 is something that probably only the publishers need worry about. But surely the price could have been kept down—for example, by omitting the colour plates and even the half-tones?

The first two-fifths of the book deal with the non-nervous physiology. After an introduction touching on the history of cephalopods, comes an "outline of the anatomy"; experts will regret that there is nothing new here (the diagram of the brain is a familiar one from Young and the three figures of the circulatory system are Isgrove's 70-yr-old drawings of *Eledone*) but someone who has never seen or dissected an octopus will certainly find it helpful. Then there follows a series of very interesting chapters on

respiration, circulation, excretion, digestion, reproduction and endocrinology. There is a wealth of information here, nearly all brought together for the first time, much of it in the form of line diagrams, and well-chosen graphs and tables (often most usefully combining data from more than one author). Chapter 3 includes unpublished results from Dr Wells' own experiments, confirming that the heartbeat of *Octopus* is very slow to regain its 'resting' level after quite moderate exercise. Equally interesting is his discovery that if octopuses experience sexual 'excitement' it is not manifest at the level of the circulation: no changes in heartbeat frequency or amplitude are detectable in males during copulation. These are only two of several examples that the author uses to point up his recurrent theme that octopuses are phylogenetically remote from ourselves, so that they may be expected to show features that reflect their molluscan origins rather than their vertebrate habits. Obvious to a zoologist, perhaps, but a valuable reminder to a mammalian physiologist or a psychologist, that the perceptual world and motivating systems of octopuses must be very different from our own.

In the middle fifth, which deals with the sense organs and sensory processing, this idea is pursued as the author reviews his own work on the curiously (by vertebrate standards) restricted use of proprioceptive information by *Octopus*. Vision is dealt with very fully in this section but a number of interesting points are left in the air. For example, is there a 'fovea' in the centre of the retina, as Tasaki's work and the experiments of Muntz imply? How do we reconcile behavioural evidence that *Octopus* can discriminate between two sources of polarised light when the *e* vectors lie at 45° and 135° with electrophysiological findings that this is impossible at the retinal level? And is the cephalopod retina really so "simple"? The recent experiments of Lange and Hartline suggest that functionally it may be quite complex. Of course, these are detailed criticisms but it seems important in a book like this to draw attention to areas of ignorance—indeed, Dr Wells is very clear about this later on when dealing with learning and the nervous system.

The final two-fifths of the book are devoted to these topics and also includes a chapter on motor control. It is difficult for someone working in this field to see the wood for the trees here. Undoubtedly

we must all be grateful to Dr Wells for summarising so much information, but could it not have been done with a bolder touch? In the chapter on tactile learning, for example, there is a wealth of detail that makes parts of it almost totally opaque. Happily, this is not true of the last chapter, on visual learning, which ends on a stimulating note with an account of the author's model of an associative learning system based on the connectivity of the vertical lobe.

On the whole, the balance of the book seems just right, given that we know so much more about the brain and sense organs. It is the omissions that will cause eyebrows to be raised. For example, there is no mention of jet propulsion, a most characteristic feature of this and other cephalopods. In a lengthy section on the optic gland, an alternative hypothesis that has recently been suggested for its function is completely ignored. And, most extraordinary of all, nothing is said about the physiology of nerves. The index does not include "action potential", "axoplasm", "sodium pump" or "synapse". Of course, virtually all the now classic work on cephalopod neurophysiology has been carried out in squids but these are closely related to octopuses and the work is of such coherence and importance that it seems strange to omit it altogether.

Perhaps Dr Wells was reluctant to do his homework here, but what is really called into question is the wisdom of writing a book on one species when its biology has not been comprehensively studied. A *Physiology of Rhodnius* might seem a fine idea but I suspect its author would hesitate when he came to the sense organs. What should he do then: omit the entire work of the Canberra and Tübingen schools or write a *Physiology of insects*? A daunting task indeed; but a *Physiology of Cephalopods* need only draw on data from two other genera (*Sepia* and *Loligo*) to be complete! In fact this is not a *Physiology of Octopus* at all but an account of areas of *Octopus* biology that interest the author. The result is very readable, well illustrated and likely to be a source book for some time. It is not, however, an authoritative or definitive work such as we would expect of someone like Dr Wells who has himself made important contributions to the physiology of *Octopus*. I am sorry to have to say this. But not very. □

*J. B. Messenger is Senior Lecturer in Zoology at the University of Sheffield, UK.*



## Prokaryotic cell surface polysaccharides

*Surface Carbohydrates of the Prokaryotic Cell.* Edited by I. W. Sutherland. Pp. 472. (Academic: London and New York, 1977.) £19.50; \$38.

IN his introduction, the editor of this volume says that "emphasis has been placed on topics which have received relatively little attention in review journals or in other volumes"—a creditable aim. Where it has succeeded, it has produced some very valuable reviews, such as that by Alvin Markovitz on the genetics and regulation of capsular polysaccharide formation; perhaps "radiation sensitivity in the title to this article might have been omitted. But because of the pace of advance of the biological sciences over the past ten years and the number of review journals, such an aim is not easy to fulfil.

When certain subjects have indeed not been reviewed, there may be good reasons for this. For example, Dr Roth was struggling against impossible odds in his chapter dealing with the physical structure of polysaccharides. Unfortunately polysaccharide capsules around bacteria are like the wholesome melon, mostly water; just how seriously one can take the spikes, filaments and networks of material, left to be seen under the electron microscope after the necessary dehydration of such structures, is questionable.

On the other hand the structures of lipopolysaccharides extracted from *Enterobacteriaceae* can hardly be said to have been ignored by other recent reviewers. Nevertheless they are described in three separate chapters in this book by Stephen Wilkinson, by Jann and Jann, and by Lindberg. The story is told from different viewpoints and each author introduces much other material, but perhaps a stern editor would have brought the three authors together and asked them to cross-reference their articles, only one description of the lipopolysaccharides of *Salmonellae* or of *Escherichia coli* was necessary.

Much interesting information is contained in Dr Dudman's article on the function of surface polysaccharides. But as he himself says it is really a series of small almost unrelated essays, just because too little definitive work has been done on the subject as a whole, to make a coherent review. Lindberg's article on bacteriophage adsorption and surface polysaccharides is an outstanding and valuable one, as is Stephen Wilkinson's on the structure of lipopolysaccharides; the authors cannot be wholly blamed for the overlap referred to above.

The two articles by the editor, on the formation of expopolysaccharides and

the enzymic attack on surface polysaccharides, bring together interesting work not reviewed for some years.

The bias in this book is strongly towards Gram-negative species, so much so that the ultrastructure of the cell walls of all bacteria is defined in one chapter, solely as that present in these species—enough to make any honest streptococcus turn in its capsule! Moreover in the chapter entitled "Bacterial Polysaccharide Antigens", there is no mention of cell wall polysaccharides, or of the teichoic acid antigens in Gram-positive species. Incidentally the electron microscope picture of a section of *E. coli* that appears in this chapter is perhaps not the most enlightening that could have been chosen from the myriad available.

This book is not entirely satisfactory.

## Metals in the liquid state

*Liquid Metals: An Introduction to the Physics and Chemistry of Metals in the Liquid State.* By Mitsuo Shimoji. Pp. 391. (Academic: London and New York, 1977.) £16.50.

WRITING a book which goes into detail about the nature of liquid metals is not an easy task. To begin with, though much of the subject is understood in broad terms, matters of detail are still highly controversial. An author must therefore steer a delicate course between taking 'a point of view' and reproducing uncritically a mass of experimental data and speculative theory. A second problem is that there have appeared in recent years, several books on the liquid state in general and one book (Faber's *Theory of Liquid Metals*) on liquid metals in particular. This latter book, published in 1972, contains a comprehensive review of most of the outstanding problems; there have been (with two possible exceptions) essentially no new theoretical developments since then, although a good deal of useful consolidation has gone on. The two fields in which new developments have occurred are those of liquid transition metals and the general area of surface properties.

Given these problems, I believe that the author has succeeded in writing a comprehensive account of the subject at a level suitable for graduate students and beyond. In parts, the author, in an effort to be comprehensive, has failed to give the sort of critical appraisal of the topic under discussion one expects to find in a textbook at this level. On the other hand, there is a wealth of experimental data contained in the 400 or so pages and the busy researcher will find the book invaluable

Inspection of the dates in the bibliographies together with the addendum added to one article, makes it clear that the gestation time, once the original articles were to hand, was not less than about two years. This is simply not good enough and deprives the book of much value, a great pity as it contains three or four quite outstanding chapters. A little firmer editing and speedier publishing, might easily have eliminated many of its deficiencies. Libraries might still purchase it for the excellent articles already mentioned, as these genuinely contain much work that is not reviewed elsewhere.

H. J. Rogers

*H. J. Rogers is Head of the Division of Microbiology at the National Institute for Medical Research, London, UK.*

as a major source of important references.

The book contains nine chapters, all of comparable length. The structural and thermodynamic aspects are covered in the first four chapters. The emphasis here is on the statistical mechanics of simple liquids modified to include the effect of the electron gas. The treatment is standard, but brings out rather clearly the role of the uniform background and how this modifies a simple hard sphere approach. Chapters five and seven deal with electron states and electron transport. Most of the old puzzles are referred to (for example, the Hall coefficient of liquid lead), but nothing significantly new is said about them. The newer field of liquid transition metals receives a rather briefer treatment than I expected.

I enjoyed chapter six which deals with atomic transport and I was pleased to see the problem of collective motions in liquid metals put into proper context by reference to the Copley-Lovesey criterion. The two remaining chapters deal with the metallic to non-metallic transition and with heterogeneous phases and surface properties. The inclusion of metal-salt solutions in the discussion of the metal-non-metal transition is welcome in view of the theoretical and practical importance of such systems. The review of surface physics, though useful, suffers from the fact that this is a rapidly expanding subject and inevitably, very recent, but significant work has not been included.

The author has faced the challenges of writing a book on liquid metals and has, in the main, overcome them. I can recommend this book as a useful work of reference to those physicists, metallurgists and chemists who are working in the field of liquid and amorphous metals and alloys.

J. E. Enderby

*J. E. Enderby is Professor of Physics at the University of Bristol, UK.*

## Iron metabolism

*Iron Metabolism.* CIBA Foundation Symposium 51 (new series). (Elsevier: Amsterdam, New York and Oxford, 1977.)

THE steady flow of research publications and symposia devoted to this topic illustrates the continuing fascination exerted by iron on a whole range of workers in the biological sciences—biochemists, bacteriologists, pathologists, clinical scientists, and more recently, immunologists.

This volume contains the proceedings of a CIBA Symposium held in London in December 1976 under the Chairmanship of Professor A. Jacobs. There are 16 contributions on selected aspects of iron metabolism, well chosen to cover topics of current interest and new advances over a wide scientific front. The format is conventional; most of the contributors, American and European, present authoritative reviews of the subject under consideration, usually including some additional new data of their own, following which the topic then receives open discussion. Also included are two periods of less structured general debate; firstly, on the iron-storage compound haemosiderin; and secondly, on ferritin, transferrin synthesis, and passage of iron into cells.

In a volume of such general excellence, it is perhaps invidious to select individual contributions for comment. However, the reviews on the structure and function of the storage compound ferritin by Dr Pauline Harrison, on the phenotypes of ferritin by Dr J. Drysdale, and on the iron-transport protein transferrin by Dr E. B. Brown are particularly useful statements of the present state of knowledge.

The iron-binding and transport proteins elaborated by bacteria, the illuminating analogies which can be made with the function and behaviour of mammalian iron-binding proteins, and the hope that such bacterial compounds could lead to more effective iron chelators for the treatment of human iron overload, provide a good example of the progress possible from coordination of research effort in different fields; this topic is well served by Dr J. B. Neilands, and by other contributors during discussion. A second area of common interest to clinicians and bacteriologists is the role of iron and iron-binding proteins in infection. There is strong evidence that iron availability is critical for bacterial growth, suggesting that mammalian iron deficiency might protect against bacterial infection; on the other hand evidence continues to be presented (here by Dr R. K. Chandra *et al.*) that iron deficiency may be associated with impaired immuno-

competence and therefore increased liability to infection. This problem remains to be solved, and awaits good statistical evidence on the incidence and severity of infection in iron deficient and iron replete subjects.

Included among many other important contributions are new data on the pathogenicity of iron overload in haemochromatosis and thalassaemia; on the abnormalities of haem synthesis and other intracellular events in sideroblastic anaemias; on iron kinetics and erythropoiesis; and on altered monoamine metabolism in the brains of iron-deficient rats, and the platelets of iron-deficient humans.

It is perhaps unjust to complain of the lack in this Symposium of an imaginative, entirely new general hypothesis such as the Fletcher-Huehns proposals on the functional heterogeneity of the two iron-binding sites on transferrin, now more than 10 years old; Professor Jacobs' revival of the idea of a labile intracellular iron pool probably comes closest to this. There is also a stimulating proposal by Dr R. L. Willson that anatomical or

functional 'decompartmentalisation' of intracellular iron may lead to cell death or to sublethal cellular damage with subsequent carcinogenesis, and that zinc could play a role in protecting the cell against the damaging effects of such iron.

The book is beautifully produced, with an excellent index; there are good figures and illustrations, and a list of references is appended not only to the formal contributions, but to those made during open debate as well. Perhaps a slightly heavier editorial hand might have been laid with benefit on the discussion sections, which occupy almost one-third of the book and are inevitably somewhat inconsistent in quality; however, these sections contain much that makes the book the exciting and stimulating production it is, and should not be missed.

It is a pleasure to recommend this volume strongly for all working in the field of iron metabolism.

**J. H. Dagg**

*J. H. Dagg is Consultant Physician in the University Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow, UK.*

## Geology of coral reefs

*Biology and Geology of Coral Reefs.* Vol. 4: Geology, Part 2. Edited by O. A. Jones and R. Endean. Pp. 337. (Academic: London, New York and San Francisco, 1978.) \$35; £24.85.

THIS volume came aptly to the attention of this reviewer shortly after re-visiting Low Isles, the site of the Great Barrier Reef Expedition 50 years ago. Much of its contents deal with these reefs—notably the account by O. A. Jones of the Great Barrier Reefs Province in which he discusses the vexed question of the nature of the foundations of these reefs, a subject on which, however, further light will be thrown with the publication of the results of the Royal Society and Universities of Queensland Expedition to the northern reefs. There is a useful bibliography of publications on the Great Barrier Reefs; also some reference, omitted earlier, to the relevant results of the Royal Society Expedition to the Solomon Islands in 1965.

Valuable papers by G. R. Orme deal with the Coral Sea Plateau beyond the confines of the continental shelf and with sedimentation in the coral reef environment. Much of what constitutes a reef is not the cemented mass that resists oceanic seas but the often immense banks of derived material to leeward. The nature and origin of these sediments, which are of great geological significance and provide a distinct environment for life, are

most productively analysed in what many will find a highly stimulating review.

Most readers will be closely held by the first three chapters under the impressive authorships of first Harry Ladd, and then J. A. Steers and David Stoddart. The first two papers deal with types of coral reefs and then specifically with fringing and barrier reefs and atolls. Here are surely laid to rest the long drawn out and largely illogical controversies between the believers in subsidence, in glacial control and in antecedent platforms. Darwin emerges supreme; the uplift he viewed in the South American Andes has been shown to be balanced by subsidence in oceanic areas, though it required borings for test explosions with atomic bombs to demonstrate this with final clarity. And although Daly's theories hold little water today, they did emphasise the significance of Pleistocene reefs which are so often the basis over which modern reefs form little more than a veneer.

The second paper from Cambridge is about coral reef islands, the authors discussing what James Cook in 1777 wrote about as the "different opinions amongst ingenious theorists concerning the formation of such low islands". Such sand cays may be buttressed by beach rock or sheltered by shingle mounds behind which mangroves become established.

This book, the final volume in the series on coral reefs, should prove of interest to a wide body of readers.

**C. M. Yonge**

*Sir Maurice Yonge is Honorary Research Fellow in Zoology at the University of Edinburgh, UK.*



## Enzyme biochemistry

*Chemistry and Control of Enzyme Reactions.* By K. G. Scrimgeour. Pp. 633. (Academic: London and New York, 1977.) £24.50; \$47.90.

THIS book attempts to cover, in a single volume, most aspects of the biochemistry of enzymes, from structure and mechanism to metabolic regulation, from purification to chemotherapy. The scope is laudable; the execution must, however, be faulted.

A reviewer of a book of this length (over 600 pages) ought not to have to quibble with its balance. Yet we find 30 pages devoted to the pterin coenzymes and a page-and-a-half to the principles of rapid-reaction techniques, 10 pages to the ferredoxins, and no description (apart from a reference elsewhere) of how to measure  $K_i$  for a reversible inhibitor.

Molecular enzymology is effectively a physical science. By adopting an avowedly descriptive approach, Dr Scrimgeour fails to bring out the growing precision in the study of enzyme structure and mechanism that has been the hallmark of the past 10 years of collaboration by X-ray crystallographers, protein chemists, organic chemists and kineticists. Many of the facts are there but the message is blunted.

The discursive nature of the book works better in descriptions of the biological significance and background of

the many enzymes used as examples. For example, in the chapter on "Metals and Enzymes", the account of nitrogen fixation does not ignore its economic and biological importance. Few aspects of enzymology are left unmentioned, the final chapter of the book ranging from hormone action mediated by cyclic AMP, to repression of enzyme synthesis in bacteria, and finishes with chemical theories of taste and smell.

Dr Scrimgeour achieves his aim of using straightforward English. He writes with enthusiasm and obvious affection for enzymes in all their guises. I have already pointed out my own preference for a more analytical approach. I would also have liked a more critical stance. Too often I had the impression that a published result was included without due heed being paid to its weight or reliability, though this does add to the undeniable value of the book as a source of references.

The book is nicely turned out, with few mistakes or misprints. It is too long and detailed in some areas and lacks the necessary balance for it to be commended to undergraduates or research students as a teaching book. Add to that its high price and its likely future as a source book worth dipping into for its references (comprehensive up to 1975) and its more descriptive passages becomes clear.

**Richard Perham**

*R. N. Perham is Reader in the Department of Biochemistry at the University of Cambridge, UK.*

## Topics in geography

*Contemporary Problems in Geography.* Three new volumes. (Clarendon/Oxford University Press: Oxford, London and New York, 1977.) Hardback each £6.50; paperback £3.

THE Editors of this new series (of which one volume—*Mathematics for Geographers and Planners* by A. G. Wilson and M. J. Kirkby—has already appeared) make its purpose very clear: to provide up-to-date, concise reviews of selected topics (not problems as they state) in geography, at a level suitable for first- and second-year undergraduates who have done little or no specialised geography before, and who need a broad understanding of an area which will fit in with other aspects of geography and environmental science. The three volumes under review were apparently designed also to fill the acknowledged gap in the available literature between advanced texts written

by experts for experts and to puzzle bright postgraduates, and volumes written as advanced school texts.

If the three new volumes in the series are judged by these stated criteria, only Statham's book is completely satisfactory. Trudgill's is both badly written, badly organised, repetitive and limited. Goudie's is too high powered for the audience at which it is aimed, but given certain alterations is something of a *tour de force* at a higher level.

All the books are reasonably priced: at least one publisher has apparently resisted the temptation to join the escalating price game and had the courage to issue both hardback and paperback versions simultaneously and thus price an up-to-date volume within the declining purchasing power of potential users. Furthermore, printing, layout, referencing, and so on, in almost all volumes is of the high order expected from the Clarendon Press.

The focus of *Soil and Vegetation Systems* by S. T. Trudgill (pp. 180) is the consideration of nutrient elements in soil and vegetation systems. Energy

and water flow and their cycling systems are not treated in depth; thus, the book title is misleading. The difficulties faced were the need to make bold generalisations about the whole system yet retain accuracy over specific points. The method adopted is to treat each selected component of soil and vegetation systems in turn in the hope of building up a sequential picture towards the end of the book. Unfortunately, this hope has not been achieved.

The first section of the book deals with general problems of understanding and explanation and how these may be tackled using models. In the second part of the book, four chapters deal with nutrient systems: weathering processes, atmospheric inputs, leaching and cycling. Only calcium, magnesium, potassium and silicon are treated at all fully; nitrogen, carbon, phosphorous, trace elements energy and water are not dealt with in detail. In the third part of the book, two chapters deal with some of these systems as functional entities. Overall, the author states that the book is concerned more with ideas and concepts; thus, it is neither a factual reference text nor a comprehensive treatment of what its title would suggest. The treatment is in parts most uneven, the writing turgid and the 'bold generalisations' largely absent. A certain basic knowledge of soil, weathering, vegetation and hydrology is assumed.

The layout differs somewhat from the other two books in the series, with fewer references at chapter ends, less helpful and selective comment on these, and distracting cross referencing in the chapters themselves. The chapters generally begin with the more complex notions and conclude with the simplest models; and these are not original but standard. Surely at this level, the reverse procedure should have been adopted—that is, the simple model, its underlying assumptions, its inadequacies, suggestions for its improvement, followed by discussion and conclusions.

Clearly, organic cycling and water cycling should have been dealt with. Overall the structure of the book is weak, it reads still like a set of lecture notes. And I, for one, wish that the Editor's loyalty and patience had been troubled by several more drafts than the vast number alluded to. But who these days has infinite patience and infinite time? The first bold (and unsubstantiated) generalisation appears on p35. For many sections, vastly more accurate, informed, well-expressed reviews can be cited. The text lacks authority and coherence; there is a desire to impress by the citing of numerous references, but accuracy over detail has submerged the initial stated aims.

The water cycling system is largely ignored, but admitted to be fundamental (p45): in sum the book is an amalgamation of factual accumulation and unauthoritative interpretation, structurally flawed and badly organised: as one focused on systems, there is no clear and early distinction between open and closed systems. By the time I had got half way through this book, though I read it to the end, I had already formed the conclusion that the contemporary geographical problem was with the author and his approach, not the topics under discussion. This is not a text I would recommend at university level. It does, however, contain information of value, if one has the time and patience to search for it.

The subject of *Environmental Change* by A. S. Goudie (pp244) is environmental change over the past 3 Myr and the topic is approached from a geographer's viewpoint. It aims to show how the physical environment and landscape has changed during the time that "man has lived on earth". The environmental changes focused on are varied: climate, sea level, vegetation association, desert and lake limits, river discharge variation, hurricane frequencies, sea ice cover and mammalian variations. All these changes are properly seen by the author as necessary to the understanding of present-day soil, landforms (though his timescale in relation to the latter is too short), and floral and faunal distributions (because, as is rightly stressed, many features of the present environment and landscape are not in equilibrium with present processes and cannot thus be explained by time).

The impact of man's activity forms only an incidental part of this book, this being seen rightly as justifying a further volume in its own right (perhaps an up-dated and synthesised digestion of "Man's role in changing the face of the Earth"—a mammoth task).

By far and away, this is the most exciting of the three volumes, to the professional academic geographer—not the audience for which it was designed. Fluent, impressively researched, bold in generalisation, generally correct in fact and interpretation, this book is an impressive achievement: it instructed and informed its reviewer very well in most areas. There are of course flaws in such a compressed, generally well judged and fully researched volume; but these are small and subject to counter-argument: the sparse treatment of East Africa (once the key area and still central geographically in terms of evidence); the deficiency of research into the alternatives of the 'Cambridge view'; and the lack of mastery/reading of the French sources here (and else-

where on occasion). The author must be faulted for trying to be too clever. This is not a beginning text for first-year students, but a text for researchers of arguement over. It is in many ways a *tour de force* but this is not what the series is said to be about.

Many (despite the number quoted) key references are absent. If the volume comes out in an expanded second edition, it could then be a *chef d'oeuvre*. I use the French terms intentionally, for where are references to Cahen, Lepersonne, de Heinzelin, and so on? The section on tropical Africa is particularly weak and uncritical: Olduvai (its interpretation, and so on) is not considered in detail, but is critical to the argument. Lake Chad is weakly treated but is crucial, as it is in a stable tectonic area. Carping comments these may be, but a volume of this calibre (with even greater future potential) deserves more than a few lines, and can absorb, as can its author, a few sabre thrusts.

The aim of *Earth Surface Sediment Transport* by F. Statham (pp184), on the broad subject of transport processes, is to introduce the basic mechanics and chemistry of sediment and solute transport at the Earth's surface. The book concentrates on the processes involved, rather than on their geomorphological implications—for example, in relation to drainage basin and slope form. Differences between

specific environments or different types of fluids are covered in superficial way. A conscious attempt has been made to group together processes or groups of processes according to their similarity of operation. The major groups discussed are mass movements (rapid and slow), fluid sediment transport and solute transport.

This text is in a number of ways the most satisfactory of the three under review, bearing in mind the level at which it is aimed, and the requirement to be concise, selective and well-organised. It certainly does cover a problem area in geography—the frequent lack of a simple knowledge of mechanics and chemistry among geography undergraduates.

The seven main sections of the book are organised with admirable clarity and selectivity: the mechanics and dynamics of material transport; sediment in transfer systems (its nature and properties); transfer through soils; transfer of sediment by rapid mass movement; slow mass movement processes; material transport in fluids; and process regimes and the time aspects of material transport. It thus forms an important bridging text in environmental sciences between hydrology, soil science, mechanical engineering and geomorphology.

**P. H. Temple**

*P. H. Temple is Professor of Geography at the University of Birmingham, UK.*

## Mechanical properties of amorphous materials

*Physical Aging in Amorphous Polymers and Other Materials.* By L. C. E. Struik. Pp.229. (Elsevier: Amsterdam, 1978.) \$39.95.

It is very rare that any book concerned with physical properties has one common theme running through the text. L. C. E. Struik's book is the exception to the rule in that its whole theme is devoted to showing the existence and explanation of an effect called physical ageing.

The property in question is that related to quenching an amorphous material through its glass transition temperature. The physical properties of the material such as creep, yield strength and electrical properties will then subsequently change with the time that the material is held below the glass transition temperature. The author shows that the effect is real and quite general for many different amorphous polymers and other materials such as glassy metals. The interpretation of the effect is also unified in that the

concept of free volume is used throughout.

Essentially an amorphous material quenched below its glass transition temperature  $T_g$  will initially be in a non-equilibrium state and possess a free volume greater than its equilibrium value. Because of mobility below  $T_g$ , with time the free volume will decrease towards its equilibrium value with corresponding changes occurring in the physical properties of the materials. This becomes manifested, for example, in a 20% decrease in the creep compliance over a period of three years for a polymer such as PVC.

The work presented in the book is authoritative and the experimental results have been collected from one laboratory over a period of sixteen years. It is a pleasure to discover an effect which seems to be general for a wide range of amorphous polymers, other amorphous materials such as inorganic and metal glasses, and for more esoteric materials, of which "compression moulded dry cheese powder" is an unusual example. Enthusiasts of mechanical properties and amorphous materials should be encouraged to read this book.

**M. R. Mackley**

*M. R. Mackley is Lecturer in Materials Science at the University of Sussex, UK.*



## Membrane transport

*Membrane Transport in Red Cells.* Edited by J. C. Ellory and V. L. Lew. Pp. 470. (Academic: London and New York, 1977.) £21; \$41.

THE red blood cell is a favourite experimental object for the study of transport across plasma membranes, because of its ease of procurement (one only needs a syringe and a not too accurate aim) and its simplicity (in mammals the red cell is devoid of nucleus, endoplasmic reticulum, mitochondria, and all membranes other than the enclosing plasma membrane). This extreme simplification of structure led early workers to sometimes regard the red cell as a dead cell, nothing more than a bag of haemoglobin. That such a viewpoint is untenable will become clear to anyone who reads this fine new book. The red cell is shown to be bustling with transport activity, and the secrets which can be uncovered in this simple cell are often of much more general application.

What sets this book apart from other such compilations of transport articles is the scope of the topics covered. We range from salamander to duck to dog to man, looking at cations, anions, amino acids, sugars, and even glutathione. Further, the book is not the hasty end-product of a conference but rather a carefully chosen set of original articles. And the authors are not the usual set of symposium speakers—instead, new faces in old fields, as well as experts in more neglected areas. The articles are mostly up-to-date and contain much unpublished material as well as the valuable off-the-cuff comments usually lacking in journal articles. On the whole, the approach is physiological and phenomenological rather than molecular biological, as most of the transport systems considered (apart from the sodium and calcium pumps and the anion exchanger) have yet to be isolated.

Half of the eighteen articles are concerned directly with cation transport. An opening chapter (Cavieses) on the well characterised sodium pump is followed by one (Beaugé and Lew) on the little discussed passive fluxes of sodium and potassium. It is made clear that these passive movements are not simply uninteresting leaks as was once thought, but seem to be mediated by specific membrane proteins. There follow two articles by Ferreira and Lew on passive calcium movements and the role of intracellular calcium, which includes the opening of a passive pathway for the release of potassium and thus possibly the modulation of membrane potential.

The use of genetic mutants for elucidating cation transport mechanisms in man (Wiley) and in ruminants (Ellory) is well described. An apparently vestigial system

for facilitating choline movements in human red cells is detailed by Martin. Kregenow presents a comprehensive review on the properties of avian red cells, which are nucleated. A bonus to the reader is the chapter by Parker on sodium and potassium movements across cat and dog red cell membranes. I found this the best and most lucid summary I have seen on this topic (even though my name is misspelt nine times!). These unusual cells lack the nearly ubiquitous sodium pump and have in its place both an apparently unique gated system for downhill sodium movements and a mechanism, probably linked to calcium movements, for active extrusion of sodium.

A chapter on water and small non-electrolyte transport (Sha'afi) tries to reconcile all experimental data to the paradigm of the Solomon "pore school". I found the exercise unconvincing. For example, to be self-consistent one is forced to pursue a rather dubious argument to the conclusion that carrier-mediated urea transport does not occur in human red cells. Naftalin and Holman review current ideas on sugar transport in human red cells in a comprehensive manner, leaving their more speculative new ideas to the end of their chapter. For perhaps the first time, the much neglected field of mammalian red cell amino acid transport is reviewed (Young and Ellory). The physiological function of such transport seems to be related to glutathione metabolism; and the active extrusion of oxidised glutathione, possibly as a pro-

TECTIVE mechanism, is described by Srivastava.

The properties of anion movements in red cells are intimately connected with its major physiological functions of oxygen and carbon dioxide carriage and exchange, as described elegantly and authoritatively by Hladky and Rink. The anion exchange system is beautifully discussed at all levels by Fortes, and Motais presents convincing evidence that organic anions ride on the same system. The heroic work of trying to measure directly membrane potentials by penetrating red cells with micro-electrodes is described by a pioneer in this field (Lassen); I would recommend, however, that the reader first look at the succinctly written clarifying note by Hladky.

Perhaps inevitable in a collection of articles spanning so many topics is a general lack of controversy, which may give the reader a mistaken impression of more agreement than actually exists. And although the editors did a superlative job of choosing topics and authors, more attention to indexing, uniform referencing, and improvement of the English of non-native speakers would have been welcomed. But these are small quibbles about an excellent book, which should be welcomed by all those interested in membrane transport.

W. R. Lieb

W. R. Lieb is at the Department of Biophysics, King's College, University of London, UK.

## Guide to immunological jargon

*A Dictionary of Immunology.* Edited by W. J. Herbert and P. C. Wilkinson. (Second edition.) Pp. 193. (Blackwell Scientific: Oxford, 1977.) Paperback £3.50.

Go into a room and you know at once if there are immunologists present. Eyes ablaze, fingers itching for blackboard chalk, they epitomise the scientist in passionate love with his subject. But draw closer and you will overhear a language that seems hardly out of the nursery. "PFC". "LPS?" "DNP SRBC". "CBA?" "T6." "TS?" "Thy 1.2." "anti-IJ?" "Ly 2, 3." "FCR?" "FC gamma." You didn't realise they were discussing the ability of cells from the thymus to inhibit an antibody response? Then you need to buy this book, which lists and defines these and some 1500 other pieces of current immunological jargon.

A further source of confusion to the outsider is that some key words have two meanings. "Allergy", for instance, means

any positive immune response to some, only certain unpleasant ones to others; "antigen" can mean an inducer of antibody, or of any kind of immune response, "tolerance" is used to mean clonal deletion, or alternatively to mean any kind of specific unresponsiveness; and so on. All these meanings, and many more, are included without prejudice in this dictionary.

Virtually rewritten since the first edition six years ago, it will no doubt continue to be brought up to date from time to time, thanks to the excellent idea of including a returnable suggestion page at the end. I have found a few omissions, and I object to the small size of one or two figures, but rather than list them here I am filling in my tear-out page so as to keep this excellent venture going.

The only major future improvement I can visualise would be the insertion of foreign terms (French and German especially), but that would perhaps be a different and a more expensive book. As it is, it will be tremendously useful to all para-immunologists — a group which nowadays must include almost all biologists and doctors.

J. H. L. Playfair

J. H. L. Playfair is Professor of Immunology at the Middlesex Hospital, London, UK.